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The nutrient flow system: Control of nutrient availability and measurement of ion uptake

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THE NUTRIENT FLOW SYSTEM: CONTROL OF NUTRIENT AVAILABILITY AND MEASUREMENT OF ION UPTAKE

A

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THE NUTRIENT FLOW SYSTEM:
CONTROL OF NUTRIENT AVAILABILITY
AND MEASUREMENT OF ION UPTAKE

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Abstract

An instrumentation and control system was built to maintain a constant nutrient supply to plants in flowing solution culture. The microcomputer based system controls the concentration of mineral nutrients, pH, root temperature, and water level. The nutrient ion concentrations controlled by the computer are nitrate, potassium, and ammonium. Ion-specific electrodes used as sensors are automatically calibrated before each measurement. Computer controlled valve manifolds and a 16-channel peristaltic pump mix aliquots of nutrient solution with ionic strength adjuster for improved electrode operation. A mathematical analysis of the performance of the Nutrient Flow System shows how the error introduced in system components contributes to error in measurements, and how experimental parameters affect accuracy. Results of plant growth trials are given, and statistical techniques for evaluating growth trial results are discussed. Over a sixteen day experiment with a target concentration of 1.0×10^{-4}-M NO_3^-, the variance of the concentration was 2.4×10^{-6}. The uptake over the experiment was 91.6 grams of nitrate. There was a 1.5% discrepancy between actual uptake and the uptake calculated by the system.

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Acknowledgments

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GLOSSARY

System Variables

$g(k)$, $G(z)$	Transfer functions
$n(k)$, $N(z)$	Additive noise
$n_K(k)$, $N_K(z)$	Parameter error
$p(k)$, $P(z)$	Uncontrolled input (i.e. nutrient uptake by plants)
$q(k)$, $Q(z)$	Observed State
$t(k)$, $T(z)$	Control input (target concentrations)
V_0, V_1, V_2	Voltage output for reference states x_0, x_1, x_2
V_x	Voltage output associated with the current state
x_0, x_1, x_2	Reference states
$x(k)$, $X(z)$	State variable (ion concentrations)
$e(k)$	Error signal

Parameters

c	Concentration of a replenishment solution
K	Steady-state gain
h	Width of a uniformly distributed error band
S	Slope of electrode response
σ^2	Variance
V_T	Volume of solution in the growing tank

Waveforms

$\delta(k)$	Dirac delta function
$u(k)$	Step function
$s_t(k)$, $S_t(z)$	Sawtooth wave with period T_t

Chapter 1 INTRODUCTION

The Nutrient Flow System is an instrumentation system designed to characterize the response of plants to availability of mineral nutrients. The most important determinants of plant growth are temperature and the availabilities of light, water, carbon dioxide, and mineral nutrients. Commercially available growth chambers have made it possible to control temperature, light, and carbon dioxide availability, but an apparatus to control accurately the concentrations of mineral nutrients is not commercially available. Several such systems have been custom built, but because of their limitations, none has emerged as a definitive solution to the problem.

Controlling the availability of mineral nutrients is difficult because of the complex relations between plant roots and the soil they grow in. Nutrient availability in soil is influenced by diffusion, chemical processes and microbial activity. Efforts to separate these effects from those of plant uptake often use solution culture techniques to avoid some of the complications of processes occurring in soils.

The Nutrient Flow System controls the availability of nutrients by controlling their concentration in a nutrient solution. Designing a system that controls several concentrations in a system with noisy measurements and nonlinear components is an interesting engineering problem. This thesis describes the system actually constructed, analyzes the system, and suggests directions for further analysis and design refinements.

Some background on solution culture techniques is useful to examine how different methods of solution culture have been used in the past. Sections 1.1 thru 1.4 discuss the effect of various solution culture techniques on nutrient availability.

1.1 Nonrenewed solution culture

The simplest and most widely used solution culture technique uses nonrenewed solutions. Nutrients are dissolved in water and put in a bucket with the plant. The initial nutrient concentrations in the solution are determined by the length of time for which the plants will be grown and for long growth periods can be orders of magnitude higher than soil concentrations [Nye & Tinker 1977]. Soils continually replenish nutrients removed from the soil solution by plant uptake [Epstein 1972], and roots are constantly reaching new, potentially nutrient-laden soil at an exponentially increasing rate. In contrast, root growth in a well-stirred nonrenewed solution culture will not make more nutrients available. As a result, this culture method is unsuitable for studies of the the effect of low nutrient availability for a long period. Also, because of the high initial nutrient concentrations, microorganisms are likely to immobilize significant amounts of nutrients.

The total amount of a nutrient a plant needs for a certain growth period can be estimated by multiplying the expected increase in plant mass by the percentage of the weight that is composed of the nutrient. For example, if fifty barley plants that weigh 0.020 g each are grown for 20 days and have an expected growth rate (RGR) of 20% per day then

$$\text{final weight} = (0.020\text{g})1.2^{20} = 0.77\text{g per plant.}$$

The total increase in weight of all fifty plants is 37.5 g, of which about 5% is nitrogen. The total nitrogen accumulated is 1.9 g or 0.14 moles. If these plants were grown in 50 liters of nonrenewed solution the initial concentration would have to be 2.8 mM to supply the expected growth requirements. Also, the plants may not be able to absorb the nutrients if the solution concentration and/or flow rate is too low. With adequate stirring, the nitrogen may be absorbed effectively down to concentrations of about 0.005 mM [Bloom & Chapin 1981], so the initial nutrient concentration should be about 2.81 mM. For this example, the expected concentration of nitrate vs. time is plotted in Figure 1.1.

If the growth rate is higher than expected or the initial weight of the plant is higher than expected the plants will use all the nutrients before the end of the experiment. Figure 1.1 shows that the concentration doesn't become limiting (drop below 0.005 mM) until the last day of the experiment. In the above example the rapid change in nutrient availability would cause deficiency symptoms in the plants, because they could not adjust their growth rate rapidly enough to compensate for the new nutrient supply rate [Ingestad & Lund 1979].

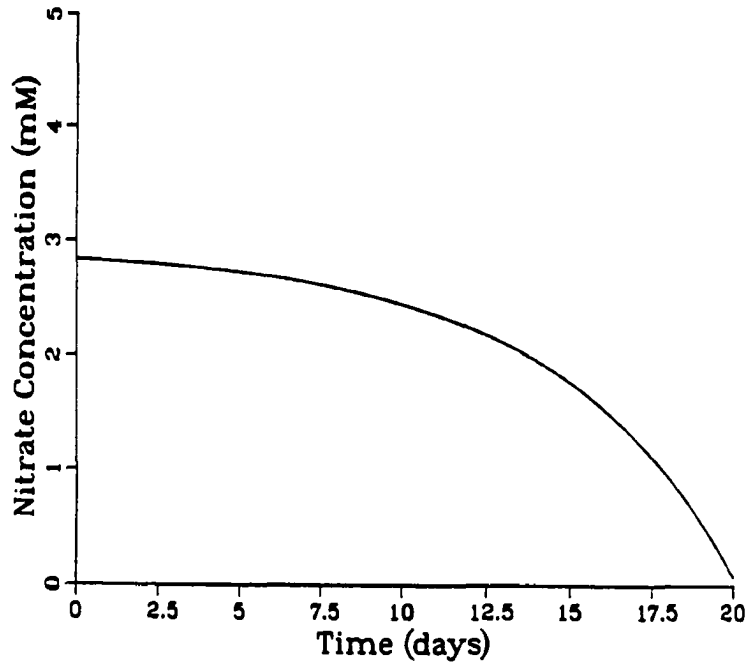

Figure 1.1 Decrease of nutrient ion concentration with time in a nonrenewed culture.

1.2 Intermittently renewed solution culture

Changing the nutrient solution occasionally in the example above allows a lower initial concentration to be used. If 1.0 mM nitrate were used initially in the example above, then

the solution would have to be changed every time the concentration reached 0.1 mM. The times at which the solution would have to be renewed would be: 14, 18, and 20 days. The nth renewal would occur at

$$t = \frac{1}{0.1823} \ln(12.6n + 1)$$

The general formula is

$$t = \frac{1}{\text{RGR}} \ln\left[\frac{n(\Delta \text{ conc})}{k} + 1\right]$$

where Δ conc = Change in concentration

$$k = \frac{(\text{nutrient to mass ratio})(\#\text{ plants})(\text{initial wt.})}{(\text{nutrient gram/mole})(\text{solution volume})}$$

$$\text{RGR} = \ln(\frac{w_{n+1}}{w_n}), \text{ Relative Growth Rate}$$

w_n = Weight of plant on day n.

The first three renewals occur at large intervals of time (Figure 1.2), but the intervals between renewals get progressively shorter until virtually continuous renewal is needed, making some sort of automated method necessary. The variance of the concentration can be made much smaller for this culture method than for the nonrenewed solution method.

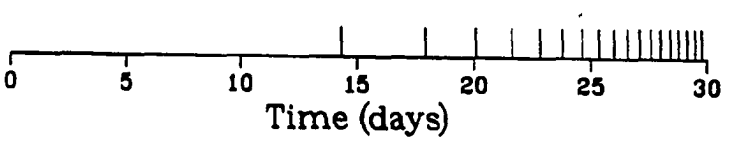

Figure 1.2 Times at which the nutrients must be replenished.

1.3 Relative addition rate method

Ingestad and Lund [1979] built an automated system that added nutrients hourly. Nutrient uptake by the plants was calculated using the formula

$$u(t) = kw_0(e^{r\Delta t/24} - 1)e^{rt/24}$$

where

$u(t)$ = uptake in moles/day.

k = the ratio of nutrient mass to plant mass.

r = relative addition rate in day^{-1}.

w_0 = initial weight of the plants.

Δt = Nutrient addition interval in hours.

An important advantage of this method over the nonrenewed or intermittently renewed methods is the ability to simulate the slow release of nutrients from the soil. This is done by making nutrients available at a lower rate than the plant's maximum capacity to use the nutrients. Plant growth is limited by nutrient concentration for the part of each period between additions that the concentration is below some critical level.

The relative addition rate method must be used with plants that are growing exponentially. When the relative addition rate method is used, the curve of concentration versus time is affected by errors in the initial estimates of the nutrient concentration of plant tissue. Another disadvantage of this method is that a long pregrowth period is necessary to use up nutrients stored in the seeds. Some large seeds have enough nutrients in them to supply the plants through the exponential period of growth so the relative addition rate method cannot be used [Ingestad and Lund 1986].

Advantages of the relative addition rate method are that precise control of the nutrient availability may be possible without concentration measurements. An unknown in this

method is how short the renewal interval must be to avoid deficiency symptoms that might result from the low nutrient concentrations that periodically occur. This may vary among plant species, reflecting adaptations to limited nutrient availability.

1.4 Constant concentration method

Steady-state conditions can be obtained in more complicated culture systems that frequently analyze the nutrient solution and replenish nutrient ions. The adjustment can be done manually, but improvements in the technology of ion-specific electrodes, computers, and electronics have facilitated development of systems that use automatic methods of analysis and correction.

Constant concentration designs provide a versatile means of simulating ecologically important environments without previous knowledge about plant nutrient status. Also, it is not necessary to use very young plants that are in the exponential stage of growth. Such designs provide the only available way to perform some experiments and reduce the labor required to perform others, justifying the increased complexity, maintenance requirements, and cost of construction.

Because automated analysis of all the nutrients in solution is impractical, the simplifying assumption is often made that plants require nutrients in constant proportions as they grow. Unmeasured nutrients must be supplied in excess of plant requirements. If they are not, there is a risk that the estimate of the proportions of some unmeasured nutrient compound required will be incorrect, and the plants will suffer deficiency symptoms. Most nutrients may vary in a hundred-fold range of concentrations in the nutrient solution without affecting plant growth much [Epstein 1972], so nutrients not of interest in a particular study are maintained at high levels.

Several approaches to building controlled concentration systems have been taken. Asher *et al.* [1965] used very large quantities of solution which flowed by plants once before being manually analyzed for nutrient content. This open-loop control technique has also been used by Bloom and Chapin [1981], Caldwell *et al.* [1978], and Reisenhauer [1969]. The open-loop technique is best suited to short-term measurements; large quantities of solution will be consumed if long-term measurements are made.

Another approach to building controlled concentration systems is to use a closed-loop control system. Clement *et. al.* [1974] built a system that analyzed some nutrient compounds and assumed that others were used in a constant proportion to the measured species. Their constant concentration design used specific ion electrodes to control autotitrators, which, in turn, controlled valves that made adjustments to the nutrient solutions. Since its original construction, the system has been expanded to measure additional ionic species using flame photometers, colorimeters and an ammonia electrode [Woodhouse 1978; Breeze *et. al.* 1982; Hatch *et. al.* 1986]. Closed loop systems have also been built by Ben-Yaakov and Ben-Asher [1982], Blom-Zandstra and Jupijn [1987], and Koch *et al.* [1987].

Our Nutrient Flow System is a closed-loop system. It was designed to take advantage of microcomputer technology to reduce the costs of construction, electro-mechanical complexity, and maintenance. Microcomputer technology allowed sophisticated control algorithms to be created and refined more easily than can be done using electro-mechanical control devices. Experimental parameters are conveniently entered into files through a menu driven user interface. Data are stored on floppy disks, so the records of nutrient additions and electrode performance data from an experiment can be easily summarized. The analysis method uses ionic strength adjuster, as recommended by electrode manufacturers [Orion 1986]. Details of the Nutrient Flow System construction follow in Chapter 2.

Chapter 2 THE NUTRIENT FLOW SYSTEM

The Nutrient Flow System (NFS) is designed to provide controlled pH, temperature, and concentrations of nitrate, potassium, and ammonium for plants growing in solution culture. The six subsystems of the Nutrient Flow System are shown in figure 2.1.

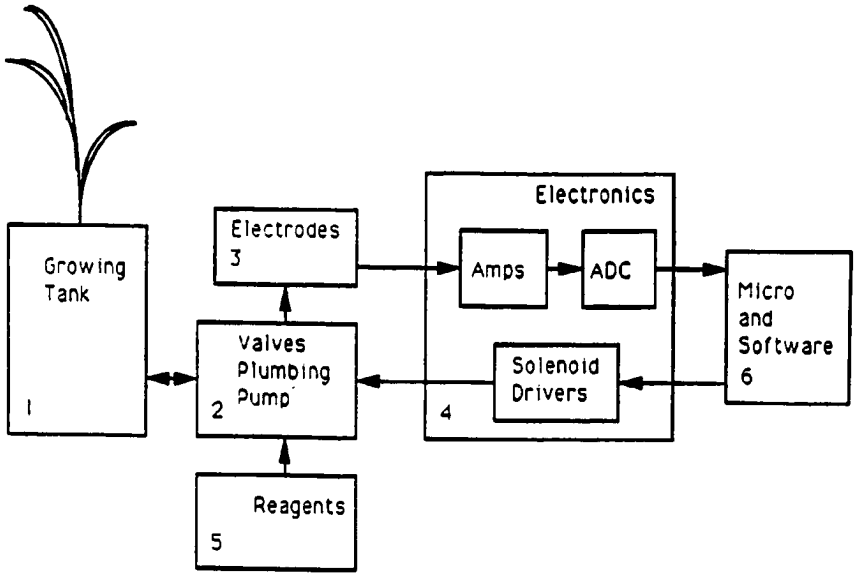

Figure 2.1 Block diagram of the Nutrient Flow System.

The growing tank provides a reservoir for nutrient solution and support for the plants. Some of the solution from the growing tank is pumped to an electrode assembly. The tank solution, and reference solutions pass through a valve manifold that selects solutions to flow by the electrodes. Electronics filter and amplify the signals from the electrodes and digitize them for the microcomputer. The microcomputer software compares the electrode

signals from the reference solutions to the signal from the nutrient solution. Based on this comparison, the software may direct the solenoid drivers to open valves that control addition of concentrated nutrient solutions. The nutrients are flushed through the plumbing into the growing tank.

2.1 The Growing Tank

Nutrient solution is held in a 30 gallon polyethylene tank. The outside of the tank is covered with aluminum foil on the sides and bottom to prevent light from reaching the nutrient solution. Nutrient solution is drawn through a screen near the bottom of the tank using a 0.32 liters per second (5 gpm) pump. (The filter screen is raised above the bottom of the tank 3 cm so that debris can accumulate at the bottom of the tank.) Solution passes at 2.76×10^4 Pa (4 psi) through 32 plastic nozzles which spray plant roots with a heavy mist. The spray system allows the solution volume to be varied to adjust the sensitivity of nutrient concentrations to the uptake rate without changing the amount of contact between roots and nutrient solution.

Plants are inserted in the opaque tank cover in small individual plastic cups with screened bottoms and perforated sides and are supported with small, chemically inert black plastic beads. The beads exclude light and are easily removed from the plant roots when plants are harvested and weighed.

A mercury contact thermometer with thermoregulator controls heating and cooling elements in the tank. Nutrient solution can be maintained at any temperature between 5°C and 25°C. The roots are constantly sprayed, so they are kept at the same temperature as the nutrient solution.

Solution level in the tank is measured by a conductivity cell. When the solution in the tank is low, it does not touch the conductivity cell. The resulting low conductivity is sensed

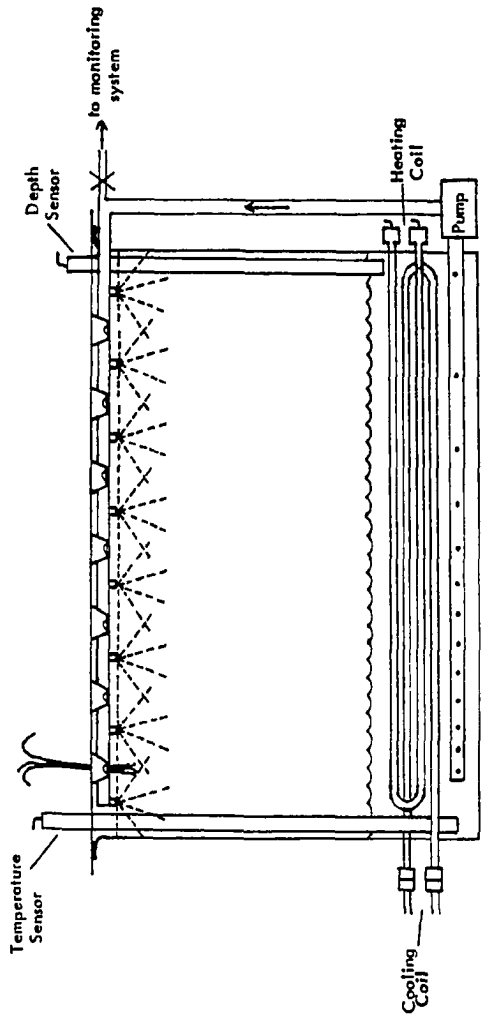

Figure 2.2 The tank in which nutrient solution is sprayed on plant roots.

by the computer program, which opens a valve to add distilled water to the growing tank until the nutrient solution contacts the conductivity cell.

The Nutrient Flow System is not limited to use of this particular type of growing tank. Any flowing culture system that is well stirred and has enough solution volume to be analyzed may be used with minor modifications.

2.2 The Valve Manifolds, Plumbing, and Pump

The nutrient flow system uses a system of valve manifolds, teflon tubing, and a peristaltic pump to route solutions. The solutions are routed as necessary to analyze the tank solution and replenish nutrients in the tank. Considerations in the design of this system were the use of proper analytical techniques, the possibility of electro-chemical interactions between components, and reduction of electrical interference.

The plumbing system:

1) Provides routing necessary to calibrate the electrodes with two reference solutions.

2) Mixes the tank and calibration solutions with an ionic strength adjuster.

3) Mixes NaOH with the calibration and tank solutions for ammonium measurements.

4) Provides undiluted tank solution for pH measurements.

5) Adds any combination of replenishment solutions to the tank.

6) Avoids the accumulation of bubbles under the electrodes.

7) Has several short sections of stainless steel tubing used for grounding points.

The solenoid operated valves (Neptune Research model 225T) have teflon valve bodies. These 3-way valves are driven by a 12 volt input and draw 130 mA. They are mounted on flat pieces of 1/4 inch PVC board and connected to each other, the pump, and reservoirs by 1/16 inch ID teflon tubing and teflon fittings.

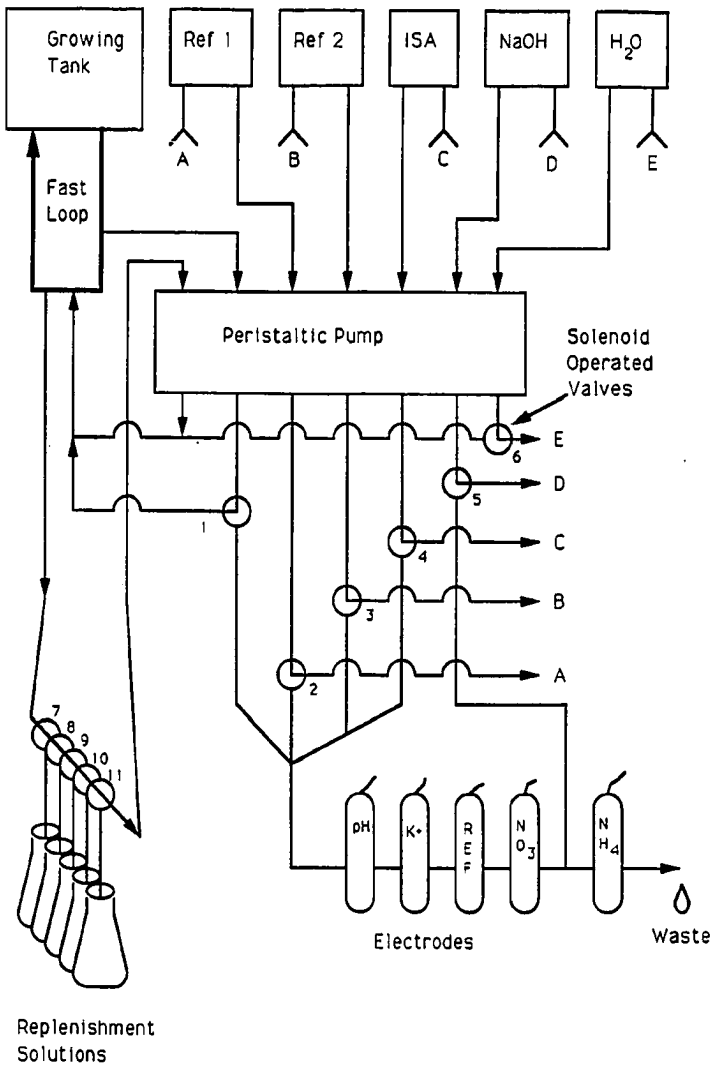

Figure 2.3 Schematic of the Nutrient Flow System plumbing system.

Solutions are pumped by a peristaltic pump (Ismatec IPN/S 16) designed for chromatography, autoanalysis, process monitoring, and other high precision applications. The peristaltic pump was used because a large number of pump channels were necessary. The tygon tubes used in the peristaltic pump contain a plasticizer that can, in the presence of other nutrients, support microbial growth. If the pump tubes are changed weekly and the nutrient concentrations in the solution are low, the microbes do not have time to flourish. Microbes only grow in the pump tubes that carry the nutrient solution from the tank. None of the other solutions include all the mineral nutrients necessary to support microbial growth. Changing the pump tubes weekly also helps maintain consistent pumping rates. (The tubes lose some of their elasticity with use.)

Figure 2.3 shows a schematic of the plumbing. The peristaltic pump must run continuously to avoid deformation of its tubes. Normally, reagents used in analysis move from their reservoirs, through the pump, through the closed position of the three-way valves in valve manifold #1 (valves 1-6) , and back into their respective reservoirs. During analysis various combinations of valves are opened to mix solutions that flow by the electrodes. The solutions are discarded after they flow by the electrodes.

Valve manifold #2 (valves 7-11) is used to add concentrated nutrient solutions (replenishment solutions) to replenish nutrients in the culture solution. The manifold is designed so that nutrient solution flushes replenishment solution (concentrated solution used to replenish nutrients used by the plants) into the tank after a replenishment has been made.

Solution circulates rapidly between the spray tank and the ion monitoring assembly outside the growth chamber. Some of this solution is removed by the peristaltic pump and directed through the valve manifolds (Figure 2.3) for analysis.

2.3 The Electrodes

Ion-specific electrodes are used to measure the concentrations of nitrate, ammonium, potassium and hydrogen ions (pH) in the nutrient solution. These electrodes are sensitive to the change in activity of ions, which depends both on the concentration of the ion they sense and the total ionic activity. The total ionic activity is a complicated function of the concentration of all the ions in a solution.

Bloom [1989] claims that ". . . failure with ion-selective electrodes derives more frequently from unrealistic expectations, procedural errors, or deficient equipment than from the inadequacies of the ion-selective electrodes themselves." The experience gained in developing the Nutrient Flow System supports this claim. Ion specific electrodes must operate in a properly controlled chemical and electrical environment. Failure to consider their limitations leads to unreliable performance.

A nutrient flow system should use the manufacturer's recommended ionic strength adjuster for each electrode. This requires an extensive system of valves, plumbing, and electrical hardware, though. Most systems take one of two shortcuts: 1) ionic strength adjuster is not used, or 2) plants are grown in the ionic strength adjuster . The result of shortcut #1 is likely to be unreliable electrode performance, while results obtained with the Nutrient Flow System indicate shortcut #2 seriously impedes plant growth. The version of the Nutrient Flow System described in this thesis uses ionic strength adjuster and mixes it with aliquots of nutrient solution for analysis. (The mixture is then discarded.)

Recent modifications have given the Nutrient Flow System ability to use a separate ionic strength adjuster for each electrode. This allows equitransferent salts to be used as ionic strength adjuster for each electrode and allows "interference suppressor solution (ISS)" and preservative solutions to be added to the ionic strength adjuster. The ISS removes

interfering ions and the preservative solution can keep bacteria from damaging electrode sensing elements [Orion 1986].

This additional capability is acquired at the cost of a substantial increase in the complexity of plumbing, number of valves, number of reagents to prepare, and complexity of software. The benefits are reduced aggravation of persons using the system and more reliable operation.

The electrode holders are specially machined from a 2 inch diameter acrylic rod (Figure 2.4). Electrodes are inserted in a hole in the top of the holder, and a seal is formed between the electrode holder and the electrode body by an O-ring clamped between the holder and a threaded fitting.

The flow cell is the part of the electrode holder where solutions come in contact with the sensing element of the electrode. The diameter of the flow cell is just large enough to cover the sensing element of the electrode, and about 1/32 of an inch deep. The low volume of the flow cell helps prevent bubbles from accumulating under the electrodes; any large bubbles are flushed out.

An advantage of having clear acrylic electrode holders is that the bubbles are visible through the holder, so their behavior in various types of flow cells may be observed. Bubbles are trapped if there is an eddy in the solution flow under the sensing element. (The centrifugal force of an eddy pulls bubbles into the center of the eddy under an electrode sensing element just as bubbles are pulled into the center of a vigorously stirred beaker.) Keeping the flow cells small results in small eddies. The holders are also tilted to help reduce accumulation of air bubbles in the flow cell.

Air bubbles decrease ion mobility between the electrodes, increasing the output impedance of the galvanic cell formed between the reference and specific-ion electrodes. Small fluctuations in electrode output occur as air bubbles flow past electrodes. If there are dissimilar metals in contact with the nutrient solution, air bubbles cause large erratic fluctuations

in electrode output. The fluctuations are a result of the air bubble causing a moving voltage divider in the tubing between the electrodes.

The electrical configuration of the system is as recommended by Bloom and Chapin [1981]. Differential amplifiers are used, with one input of a differential pair connected to an ion-specific electrode and the other to a reference electrode. The solutions are grounded by stainless steel sections in each tube between the pump and the electrodes. The ground prevents the drifting of the differential inputs and prevents static charges generated by the peristaltic pump from interfering with the electrodes. (The tubes in the peristaltic pump apparently generate substantial static charges as they pull apart after being squeezed together by the pump rollers.)

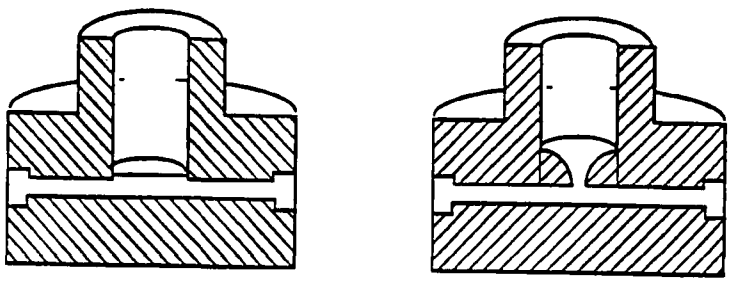

Figure 2.4 Ion-specific and reference electrode holders.

Ion specific electrodes and a reference electrode produce output voltages proportional to the logarithm of the activity of the ion they are designed to sense. They have output impedances ranging from 1 to 200 megohms. Temperature changes cause the output voltage

to vary 2% per °C. Electrodes are subject to interference from chemical sources, electromagnetic fields, and electrochemical reactions. Output voltages are also affected by flow rates of solutions passing the electrodes. Ideally, steady-state output voltage of an ion-specific electrode is given by the Nernst equation [Orion 1986].

$$V = V_0 + S\log(A).$$

Where:

V=Measured electrode potential.

V_0=Reference potential.

S=electrode slope (about 56 mV per decade concentration)

A=Activity of the measured ion.

The electrode calibration curve depends on the total ionic strength of the solution. The nutrient solutions are mixed with ionic strength adjuster (ISA) to maintain a high, constant, total ionic strength. The changes in the concentration of the nutrient ions then cause only very small changes in the total ionic strength of the nutrient solution. This prevents concentration changes from affecting the electrode calibration curve. Further references to concentration in this thesis should be understood to mean concentration derived from a measurement of activity.

Figure 2.5 shows the possible electrical connections between an electrode in a beaker and an ideal pH meter (one with infinite input impedance). To avoid errors either R_2 or R_3 must be very large and the other small. Also, R_1 must be much larger than R_4, the internal resistance. The Nutrient Flow System is configured so that R_3 is much larger than R_2. The resistance R_2 is small to prevent buildup of static electricity on the input to the MOSFET operational amplifier.

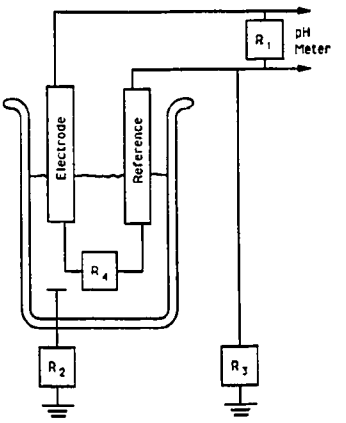

Figure 2.5 Electrical connections between an electrode, pH meter, and ground.

If there is a low resistance path for current to flow between the reference electrode and some other conductor that touches the solution, redox reactions may transfer ions into the salt bridges in the reference electrode. The transferred ions can precipitate and clog the salt bridge, causing poor electrode performance. Another effect of improper grounding is that bubbles moving through the tubes will cause large variations in the output voltage of the electrode as they move. The bubbles create a moving voltage divider between the incompatible grounds.

The best way to avoid these problems is to connect the reference electrode to one side of a high-impedance differential amplifier. This configuration requires that the solution be grounded independently of the electrodes. Grounds may be placed at several points to eliminate noise from several sources if they are all of the same (corrosion resistant) metal, so that reactions between the grounds will not contaminate the solution. Using this technique allows several electrode pairs to be used without electrical interference between the reference electrodes [Bloom & Chapin 1981]. This technique uses a smaller sample

for analysis, requires less plumbing, and has less chance of introducing air bubbles than isolation by dripping solution into an isolated electrode system [Hatch *et al.* 1986].

Ammonium is measured by an electrode (Orion) that measures the amount of ammonia gas that diffuses through a permeable membrane. Ions cannot cross this membrane [Orion 1986], so the electrode is electrically isolated from the nutrient solution and the reference electrode for the ammonia electrode should be grounded. The membrane leaks sometimes, so an undependable electrode may be an indication of a damaged or improperly installed membrane.

Ammonium is converted into ammonia gas by mixing solutions with 0.2-M NaOH. A side effect of this mixing process is that insoluble hydroxides of of other ions in the nutrient solution precipitate in the tubes, mixing coil, and electrode holder. Occasional flushing with 0.5-M sulfuric acid is required to clean this part of the system.

A flat combination electrode (Markson) with a fairly large sensing area is used to measure pH. The electrode was reliable, but a large sensing area results in a large change in cross-sectional area between the tubing and the electrode holder. Resulting eddies increase the holdover from previous solutions and provide a place for air bubbles to stop.

Like Woodhouse [1978], I found that the potassium electrode only worked for about two weeks at a time. Bacteria grew on the sensing element and damaged it. The NFS has been redesigned to allow each electrode to use a different ionic strength adjuster. Putting preservative in the ionic strength adjuster for the potassium electrode may make it last longer.

The nitrate electrode was quite reliable, but changes in the pH of the nutrient solution affected its output and increased the time required for it to stabilize (because an equitransferent ISA was not used.) This problem was overcome by including a sodium phosphate buffer in the ionic strength adjuster. The nitrate electrode output is affected by phosphate,

but the phosphate concentration in the ionic strength adjuster was constant. Accurate measurements in the presence of interfering ions can sometimes be obtained if both standards and and samples are treated similarly [Orion 1986].

2.4 The Electronics

The output from the ion-specific electrodes is weak and noisy, so it must be buffered, amplified and filtered. The electrodes have internal resistances of up to 200 megohms, so an amplifier with a very high impedance input is required. The voltage follower input stages in the nutrient flow system amplifiers have input impedances of approximately 1.4×10^{12} ohms, so the error from impedance mismatch is negligible.

After impedance buffering, the signal is filtered and amplified. Filtering is necessary because electromagnetic fields from power lines, electric motors, and other equipment induce voltages in the electrodes that make readings inaccurate. The ion-specific electrodes require up to 2 minutes to reach 99% of their steady state output value (their dominant time constant is about ten seconds.), so a filter with a very low cutoff frequency can be used to eliminate noise without distorting the measured electrode response. The anti-aliasing filters in this system have 3 dB corner frequencies of 3.3 Hz. The low cutoff frequency reduces the need for digital filtering, leaving the computer free for other tasks.

Amplification matches the output range of the electrodes to the input range of the analog-to-digital converter for improved accuracy. Outputs from the electrodes range from -250 millivolts to +300 millivolts. Amplification by 10 changes this to a range of -2.5 volts to +3 volts. The analog-to-digital converter has a range of -10 volts to +10 volts.

The amplifier should have a reasonably high common mode rejection ratio to reduce errors caused by common changes in the voltage levels of the two electrodes. Common mode

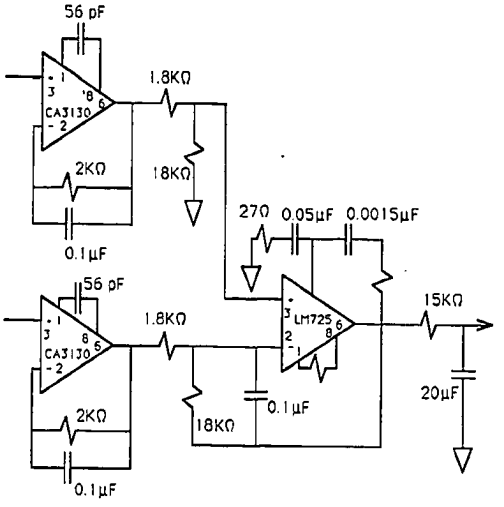

Figure 2.6 Schematic of an electrode amplifier.

noise can be caused by redox potentials with other metals in the system, electromagnetically induced noise, and static charges.

A Tecmar Labmaster I/O system converts the filtered and amplified signal to a 12 bit number. The analog to digital converter (ADC) is configured for a resolution of 4.9 mV and a range of -10 V to $+10$ V. The amplifiers multiply the electrode output ten times, so the resolution referred to the output of the electrodes is 0.49 mV.

2.5 The Reagents

The reagents used with the Nutrient Flow System are prepared from the following stock solutions; 0.1-M HNO_3, 0.1-M KNO_3, 1.5-M $NaSO_4$, 12-M NaOH, 0.1-M NH_4OH, 0.5-

M NaH_2PO_4, a macronutrient solution, micronutrient solution, and a solution of chelated iron. All solutions are normally prepared with double distilled water from a glass still. Any number of mysterious problems may arise if contaminated water is used. Tables 2.1 and 2.2 give the composition of the macronutrient and micronutrient solutions. The names "macronutrients" and "micronutrients" reflect the relative amounts of ions used by the plants. Separate solutions are used because some combinations of ions in the nutrient solutions are only slightly soluble, so they will precipitate in the concentrations present in the stock solutions.

Table 2.1 Chemical composition of the macronutrient stock solution (MACRO).

Nutrient	Concentration (moles/liter)	Form added
K^+	0.0910	KNO_3, KH_2PO_4, K_2SO_4
PO_4^-	0.0225	KH_2PO_4
SO_4^-	0.0127	K_2SO_4
Ca^{+2}	0.00873	$Ca(NO_3)_2 \cdot 4H_2O$
Mg^{+2}	0.0175	$Mg(NO_3)_2 \cdot H_2O$
NO_3^-	0.0893	KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$

One liter of macronutrient solution is prepared from 3.72 g KNO_3, 3.92 g KH_2PO_4, 2.06 g $Ca(NO_3)_2 \cdot 4H_2O$, and 4.49 g $Mg(NO_3) \cdot H_2O$. One liter of micronutrient solution is prepared from 0.616 g $MnSO_4 \cdot H_2O$, 0.570 g H_2BO_3, 0.41 g $CuCl_2 \cdot 2H_2O$, 0.64 g $ZnSO_4 \cdot 7H_2O$, and 0.008 g $Na_2MoO_4 \cdot 2H_2O$. These stock solutions are based on those of Ingestad [1971].

Table 2.2 Chemical composition of the micronutrient stock solution (MICRO).

Nutrient	Concentration (moles/liter)	Form added
Mn^{+2}	0.00364	$MnSO_4 \cdot H_2O$
BO_3^{-3}	0.00922	H_2BO_3
Cu^{+2}	0.000241	$CuCl_2 \cdot 2H_2O$
Cl^-	0.000481	$CuCl_2 \cdot 2H_2O$
Zn^{+2}	0.000223	$ZnSO_4 \cdot 7H_2O$
Na^+	0.0000661	$Na_2MoO_4 \cdot 2H_2O$
MoO_4^{-2}	0.0000331	$Na_2MoO_4 \cdot 2H_2O$
SO_4^-	0.003863	$MnSO_4 \cdot H_2O$, $ZnSO_4 \cdot 7H_2O$

The reagents for a particular experiment are prepared from the above stock solutions. Table 2.3 gives an example recipe for solutions necessary to grow plants in 25 l of nutrient solution with 0.05 mM nitrate and 0.05 mM ammonium.

Table 2.3 Recipes to make nutrient solutions from stock solutions.

Solution & Volume	Stock Solution (ml) HNO_3 0.268–M	NH_4OH 0.190–M	MACRO	MICRO	IRON	NH_4NO_3 0.10–M	KOH 0.10–M
Tank 25l	2.33	6.6	7.0	0.7	0.7	–	–
4l Ref 1	–	–	–	–	–	1.5	0.8
4l Ref 2	–	–	–	–	–	2.5	1.2
0.5 l Rep 1	–	–	50.0	–	–	–	–
0.5 l Rep 2	50.0	–	–	1.4	1.4	–	–
0.5 l Rep 3	–	50.0	–	1.4	1.4	–	–

Ionic strength adjuster is prepared by diluting 53.5 ml 1.5-M Na_2SO_4, 8 ml 0.5-M NaH_2PO_4, and 21 ml NaOH with distilled water to 4 l.

2.6 The Computer and Software

The computer and software are used to control the valves, calculate a calibration curve for the electrodes, calculate concentrations using the electrode output, and record the concentration and amount of nutrients added on a floppy disk. Other functions necessary for convenient operation of the system are a manual control mode for testing, and form screens for setting parameters (parameters include reference solution concentrations, replenishment solution concentrations, electrode performance criteria, solution volume in reservoirs, volume of solution in the spray tank, etc.)

The software that controls the Nutrient Flow System is an 1100 line Microsoft BASICA program. High level functions for manipulating the Nutrient Flow System are constructed from a set of subroutines that provide the basic tools necessary. These basic subroutines perform the following functions:

1) Display menus.

2) Create a new program disk.

3) Initialize the hardware.

4) Plot the output from an electrode.

5) Position text precisely on the computer screen.

6) Perform analog to digital conversion.

7) Turn valves on or off.

8) Set experimental parameters.

9) Read and write experimental parameters.

10) Control keyboard input.

11) Calibrate an electrode.

12) Invert a matrix.

13) Multiply a matrix.

These subroutines provide a set of tools used to build subroutines used to perform higher level operations including:

1) Read reference output # 1 from electrodes.

2) Read reference output # 2 from electrodes.

3) Read output from tank solution.

4) Clear ionic strength adjuster from the plumbing.

6) Add replenishment solution to the tank.

7) Calculate concentration and concentration error.

8) Calculate how much replenishment solution to add to the tank.

9) Calculate how much was actually added to the tank after an addition.

10) Wait for a specified period and plot electrode output on the screen while doing so.

11) Record time, date, concentrations, and replenishments in a disk file.

The high level subroutines above are combined to create short programs for automatic control of nutrient concentrations and manual control of the system for testing.

The procedure followed by the software to control concentration begins with calibrating the electrodes. Two-point electrode standardizations are performed by switching between two calibration solutions via solenoid valves 2 and 3 (Figure 2.2). Calibration solutions are mixed with an ionic strength adjuster and pass by the pH, potassium, reference, and nitrate electrodes. Then 0.2-M sodium hydroxide is added and mixed with the solution to convert the ammonium ion to gaseous ammonia. An ammonia sensing electrode measures

the ammonia concentration. A calibration curve is calculated for each electrode except the pH electrode by solving the following equations for V_0 and S:

$$V_1 = V_0 - S \log x_1$$

$$V_2 = V_0 - S \log x_2$$

where

V_1= Output voltage obtained using reference solution # 1

V_2= Output voltage obtained using reference solution # 2

V_0= Offset voltage of calibration curve.

S= Electrode slope in millivolts per decade concentration change.

x_1= Concentration of reference solution # 1

x_2= Concentration of reference solution # 2

For the pH electrode, the value of S is assumed to be 56 mV/decade of concentration, so only V_0 is found. If the calibration curves fall in an acceptable range, the nutrient concentrations in the culture solution are calculated. Otherwise the calibration procedure is repeated.

After the culture solution concentrations have been determined, concentrated nutrient solutions may be added to restore ion concentrations to initial values. These solutions are added one at a time through valves 7-11 (Figure 2.2). The concentrations of replenishment solutions are chosen to allow desirable addition times (15 to 30 seconds), and are usually about ten times the concentration of the tank solution. The following equations are solved to find the amount of each replenishment solution needed to correct an error in the concentration of nutrients.

$$e_{NO_3} = v_1 c_{1,NO_3^-} + v_2 c_{2,NO_3^-} + v_3 c_{3,NO_3^-}$$

$$e_{K^+} = v_1 c_{1,K^+} + v_2 c_{2,K^+} + v_3 c_{3,K^+}$$

$$e_{NH_4^+} = v_1 c_{1,NH_4^+} + v_2 c_{2,NH_4^+} + v_3 c_{3,NH_4^+}$$

where

e = moles of a particular ion missing from the solution culture.

$c_{i,X}$ = Concentration of a nutrient ion, X, in replenishment solution i.

v_i = Volume of replenishment solution i to add to the culture solution.

In matrix notation this can be expressed as

$$\begin{pmatrix} e_{NO_3} \\ e_{K^+} \\ e_{NH_4^+} \end{pmatrix} = \begin{pmatrix} c_{1,NO_3^-} & c_{2,NO_3^-} & c_{3,NO_3^-} \\ c_{1,K^+} & c_{2,K^+} & c_{3,K^+} \\ c_{1,NH_4^+} & c_{2,NH_4^+} & c_{3,NH_4^+} \end{pmatrix} \begin{pmatrix} v_1 \\ v_2 \\ v_3 \end{pmatrix}$$

or more compactly as

$$\vec{e} = \mathbf{C}\vec{v}.$$

The Nutrient Flow System finds the volume of each nutrient solution needed by using

$$\vec{v} = \mathbf{C}^{-1}\vec{e}$$

where $\mathbf{C}^{-1}$ is the inverse of $\mathbf{C}$. The volume, v_i, is multiplied by the pumping rate of the peristaltic pump to find the time for which the valves must be opened.

As mentioned before, the plants use nutrients in fairly constant proportions as they grow. Thus the expected values of $\vec{e}$ form a subspace of the possible values of $\vec{e}$. The matrix $\mathbf{C}$ is formed of the concentrations of nutrients in three replenishment solutions, i.e. $\mathbf{C} = (\vec{c}_1\ \vec{c}_2\ \vec{c}_3)$. The choice of compositions of the three replenishment solutions is in part determined by the consideration that linear combinations of $\vec{c}_{1-3}$ must span the space of expected values of $\vec{e}$ (put another way, $\vec{c}_{1-3}$ must be chosen so that there is some $\alpha_1, \alpha_2, \alpha_3$ that will satisfy

$$\vec{e} = \alpha_1\vec{c}_1 + \alpha_2\vec{c}_2 + \alpha_3\vec{c}_3$$

for all $\vec{e}$ in the subspace of expected error vectors.) All the elements of $\vec{v}$ must be positive; it is not possible to remove replenishment solution once it has been mixed with the culture solution.

The pH of the culture solution is adjusted after nutrient concentrations have been corrected, because replenishment of some nutrients may affect the pH of the solution. Additions of acid or base as required are used to adjust pH. The hydrogen ion concentration is not included in the calculation of replenishment volumes because the pH buffering effect of some nutrient ions would make the results inaccurate.

If no replenishments are necessary the software waits for 45 minutes before repeating the calibration, measurement, and replenishment cycle. If replenishment is necessary, the concentration, replenishment, and electrode slope for each ion is recorded and the concentration is rechecked after 7 minutes (enough time for the nutrients to mix thoroughly in the tank.) Sometimes an electrode gives erratic readings and the slope calculated is far from the theoretical value. In these cases no addition is made and the electrode is recalibrated after 7 minutes. During these operations, the output from each electrode is plotted on the computer monitor every 5 seconds to allow the operator to assess the system performance.

In addition to making repeated corrections to concentrations of nutrients in the culture solution, the software checks the level of solution in the spray tank. If this level falls below the conductivity cell, valve 6 is opened to add water until the solution level again reaches the conductivity cell used as a set-point level gauge.

The manual mode of operation allows control of the valves using keys on the PC while electrode output is plotted on the screen. Calibration curves for the electrodes can be calculated semi-automatically. The manual mode is useful for testing the system before using the automatic mode. It is also frequently used to measure the concentration of solutions from small beakers that individual plants are grown in. (These measurements are necessary to determine variation of uptake rates among individual plants, so that a

confidence interval may be placed about the mean uptake rate for plants grown in the culture tank.)

Other programs used with the Nutrient Flow System plot the uptake and concentration versus time, plot diurnal uptake, and use an electronic balance to measure pumping rate of the peristaltic pump.

2.7 Operation of the Nutrient Flow System.

The first step in beginning an experiment with the nutrient flow system is to prepare the reference solutions, ionic strength adjuster, and nutrient solutions according to a recipe like that of table 2.1. The nutrient solution is made in the spray tank, and the others are mixed in a 4 l beaker and poured into opaque eight liter plastic bottles. Replenishment solutions are mixed in batches of 500 ml and put in 1.0 liter erlymener flasks covered with Parafilm®. The volume of each solution should be recorded on a log sheet. Any time new solution is prepared and added to the system it should be recorded on a log sheet for the experiment, for confirmation of uptake calculated by the computer.

After preparing the solutions, the nutrient flow system is assembled and tested. If the tank has been removed from the growing chamber for cleaning, it is moved back into the chamber and the glycol cooling coils are connected to the chilled glycol source. The spray pattern from nozzles in the tank is checked, and any clogged spray nozzles are replaced. The three teflon tubes that connect the tank to the monitoring system outside the growing chamber are then connected. The solution flows from the tank to the monitoring system in the tube marked "out." The other two tubes are used for addition of concentrated replenishment solution and return of solution from the rapid circulation loop.

The conductivity cell that is used as a depth gauge is inserted into the hole in the spray tank cover marked "level gauge," and the screws that tighten the O-ring that holds

the level gauge in place are tightened. The conductivity cell is adjusted so it just touches the top of the nutrient solution.

The thermometer probe is similarly installed in the hole marked "temp. probe" and plugged into the temperature controller. The heating and cooling outputs from the temperature controller are connected to a stainless steel heating element and a solenoid valve in the glycol loop, respectively.

Electrodes are tested according to manufacturer's instructions, then installed in the electrode holders. Table 2.4 summarizes the connections for the electrode outputs.

Table 2.4 Electrode to Amplifier Connections.

Amplifier Channel	Input
0	Reference electrode output.
1	Nitrate electrode output.
2	Grounding short.
3	Potassium electrode output.
4	pH electrode.
5	pH electrode reference.
6	Grounding short.
7	Ammonium electrode output.
Ground	Ammonium electrode reference output.

The NFS program disk is put into the computer and the computer is turned on. The program is automatically loaded and run, bringing up the NFS menu. Concentrations and volumes of all the reagents, the sampling period, the tolerances for electrode performance, the hysteresis, the pumping rates, and other parameters are entered into the parameters files, guided by menus and form screens. After the parameters have been entered the program is put into the manual control mode.

To test the pumping rate of the replenishment solutions, distilled water is pumped from a beaker into a 10 ml graduated cylinder. To do this, the tubes to the replenishment solution reservoirs are placed in a beaker of distilled water.

The peristaltic pump tubes are replaced and tightened three clicks beyond the point when the pump begins sucking solution through the tubes. The manual mode of the nutrient flow system program is used to open valves and test the pumping rate of each pump channel and combinations of pump tubes. The results of one such test are recorded in table 2.5.

Table 2.5 *Table used to record flow rate data.*

v1	v2	v3	v4	v5	v6	v7	v8	Rate*	Solutions
x†								4.57	reference 2
	x							4.47	ISA
		x						4.64	reference 1
			x					4.69	Tank
				x				4.83	NaOH
					x			4.57	replenish 6
						x		4.54	replenish 7
							x	4.56	replenish 8
x	x			x				13.45	R2,NaOH,ISA
	x	x		x				13.22	R1,NaOH,ISA
	x		x	x				13.41	Tank,NaOH,ISA

†Indicates valve is in the open position.
*Flow rate in milliliters per minute (Data from 1/8/86).

The automatic control feature is then chosen from the program menu. The electrode outputs are displayed on the computer screen during the calibration and measurement cycle. The calibration and measurement results are printed. These are checked to see that the expected values were obtained. If the system worked correctly, the replenishment

Chapter 3 SYSTEM ANALYSIS

The performance of the Nutrient Flow System is measured by 1) the variance of the nutrient concentrations from the target concentrations and 2) the error in the uptake measurements. The purpose of this chapter is to gain insight into how the components of the Nutrient Flow System affect its performance.

Ecologists want to use the system to test hypotheses about adaptations to limited nutrient availability. Knowledge of the performance of the Nutrient Flow System is needed to determine the power of an experimental design to test a particular hypothesis.

3.1 Methods of Analysis

The responses of the electrodes are nonlinear, but the concentrations of the nutrient ions do not vary significantly, so linear models about the operating point are used to calculate electrode output.

Most components of the Nutrient Flow System produce outputs that do not depend much on past inputs or rates of change of inputs, so they can be modeled simply as proportional constants with possibly some noise added. The one exception is the growing tank. The concentration of each ion in the growing tank is a function of all past inputs of nutrients from the replenishment system and all past uptake by the plants.

The Nutrient Flow System is operated with a "deadbeat" control algorithm. The concentration is ostensibly returned to its target value after each measurement; noise in the system prevents this from actually occurring. Multiplicative noise introduced by uncertainty in the pumping rate of replenishment solutions, the concentration of the nutrient

solution tubes are taken out of the distilled water used for testing and put into beakers of replenishment solution.

While the system is operating, its operation is checked twice a day to see if any irregularities in electrode performance are developing or if any of the reagents needs to be replaced. The flow of solution in the tubes is observed to make sure no constrictions have occurred. The peristaltic pump tubes are changed once a week. Aliquots of nutrient solution may be taken daily for independent analysis. These aliquots are preserved by adding 1 ml of 1-M boric acid to 100 ml of samples [Orion 1986]. Aliquots of standards may also be taken and treated similarly.

After the experiment is completed and the plants have been harvested, the glycol quick-connectors are disconnected and the growing tank is moved out of the growth chamber for cleaning. A sump pump is used to remove nutrient solution from the tank. The electrodes are removed from the holders and rubber stoppers inserted in their place. Tubing is cleaned with 1-M sulfuric acid, then the tank and tubes are cleaned with a dilute solution of clorox (sodium hypochlorite) and finally rinsed several times with distilled water.

replenishment solutions, and the total volume of nutrient solution changes the characteristic equations of the system so that it is not exactly a "deadbeat" system. Additive noise, the dominant noise source, is introduced by components including the electrodes and the analog to digital converter (ADC).

Different techniques are required to evaluate the effects of additive and multiplicative noise. Additive noise sources may be treated as independent inputs to the system, and superposition applies to the resulting outputs in a linear system. Multiplicative noise is evaluated by finding the sensitivity of the system transfer function to variations in each parameter and multiplying by the noise to produce an error term. If the operating point of a subsystem does not vary much, multiplicative noise may be approximated by additive noise.

To evaluate the effects of additive noise, the additive noise introduced in each subsystem in the block diagram (Figure 3.1) was found. For each, there were one or more sources of additive noise, and these were combined to find the equivalent noise at the output of the block. The additive noise sources were treated as independent inputs to the entire system function.

To evaluate the effects of multiplicative noise on the system, the entire system function was calculated in the Z-Domain with the parameters carried through the calculations explicitly. Then the partial derivatives of the system transfer function with respect to the parameters were found and used as sensitivities. The sensitivities were multiplied by the Z-transforms of the multiplicative noise in each parameter to give noise terms for variation of each parameter.

Alternative methods of evaluating the multiplicative noise would have been to 1) include it as part of the transfer function or 2) approximate it by additive noise and include it in the independent noise inputs. Method 1) would have resulted in more complicated subsystem transfer functions, and thus a much more complicated system function. The distinction

between the noise and the system would have been less clear. If alternative 2) had been chosen, the effect of parameter variations on the frequency response of the system could not have been found. The frequency response affects how noise introduced by the additive noise sources is attenuated. The parameters (steady-state gains of the transfer functions) also affect the steady-state error of the system.

Two simplifying assumptions were made: 1) the nonlinear electrode response may be approximated by linear models around the operating point when calculating error propagation and 2) nutrients may be both added and removed from the growing tank. The first assumption causes small errors in the estimated measurement error. For example, if the concentration of an ion is high by 5%, the linear approximation will estimate that it is high by 4.65%, an error of 7% in the estimated error.

The second assumption may cause an underestimation of error in concentration control. If an electrode reading is low, and too much replenishment solution is added, the concentration must be corrected by plant uptake. The time this takes depends on the uptake rate, but in any case is longer than the time it takes to increase the concentration.

3.1.1 The Functional Block Diagram

The block diagram (Figure 3.1) shows the relationships among the various subsystems of the Nutrient Flow System (NFS). The lines that connect the blocks map the flow of information in the form of electrical signals, ion concentrations, and digital numbers.

The inputs are $t(k)$, the target concentration, and $p(k)$, the uptake by plants. The outputs are $q_u(k)$, the observed uptake, and $q_c(k)$, the observed concentration. The transfer functions $g_r(k)$, $g_t(k)$, $g_c(k)$ and $g_u(k)$ give a relationship between the input and output of each NFS subsystem.

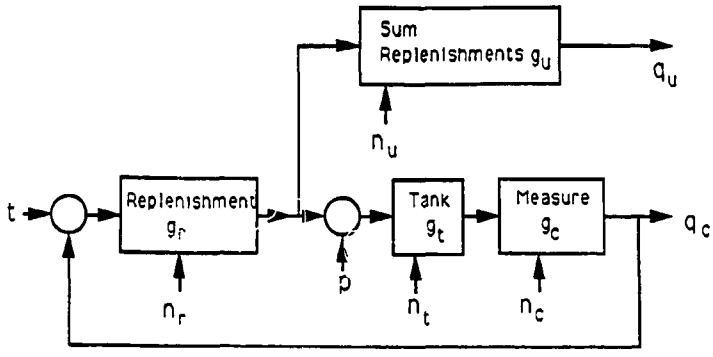

Figure 3.1 Block diagram of the Nutrient Flow System.

3.1.2 The Z-Transform

Z-Transform analysis is a standard method of analyzing linear time-invariant (LTI) systems. Differential or difference equations are required to describe LTI systems that have non-random noise, integrating elements, or differentiating elements; otherwise straightforward techniques may be used. These are used for parts of the NFS analysis where possible; otherwise, Z-transform methods are used.

The Z-Transform is a method of transforming difference equations into polynomials, which are usually more easily manipulated than difference equations. Z-Transform analysis is described in detail in many texts on control systems and signal processing [Kuo 1982; Oppenheim, Willsky & Young 1983; O'Flynn & Moriarty 1987]. The definition of the two-sided Z-transform of a function, $f(n)$, is

$$\mathcal{Z}\{f(n)\} = \sum_{n=-\infty}^{\infty} f(n)z^{-n}.$$

3.1.3 Propagation of Random Uncorrelated Error

The steady-state output of an ideal element of a linear system can be described by

$$O = KI + a.$$

Where:

O = output from system element

K = gain of a system element.

I = input to the system element.

a = a constant.

A non-ideal system may be modeled by adding additional terms to describe nonlinearity $N(I)$, Multiplicative noise inputs I_M, and interfering (additive) noise inputs I_I. An equation to describe the non-ideal behavior is [Bentley 1983]

$$O = KI + a + N(I) + K_M I_M I + K_I I_I$$

In many cases the parameters may be represented by a statistical model. The variance of the static output error, σ^2, may be found using

$$\sigma^2 = \left(\frac{\partial O}{\partial K}\right)^2 \sigma_K^2 + \left(\frac{\partial O}{\partial I}\right)^2 \sigma_I^2 + \left(\frac{\partial O}{\partial I_M}\right)^2 \sigma_{I_M}^2 + \left(\frac{\partial O}{\partial I_I}\right)^2 \sigma_{I_I}^2 + \ldots$$

The error in the output of a complete system may be calculated by using the output from one system element as the input to the next and calculating the variance using the rules for combining the effects of several gaussian distributions.

$$\sigma_{I_2}^2 = \sigma_{O_1}^2 = \left(\frac{\partial O_1}{\partial I_1}\right)^2 \sigma_{I_1}^2 + \left(\frac{\partial O_1}{\partial I_{M_1}}\right)^2 \sigma_{I_{M_1}}^2 + \left(\frac{\partial O_1}{\partial I_{I_1}}\right)^2 \sigma_{I_{I_1}}^2 + \left(\frac{\partial O_1}{\partial K_1}\right)^2 \sigma_{K_1}^2 + \ldots$$

$$\sigma_{I_3}^2 = \sigma_{O_2}^2 = \left(\frac{\partial O_2}{\partial I_2}\right)^2 \sigma_{I_2}^2 + \left(\frac{\partial O_2}{\partial I_{M_2}}\right)^2 \sigma_{I_{M_2}}^2 + \left(\frac{\partial O_2}{\partial I_{I_2}}\right)^2 \sigma_{I_{I_2}}^2 + \left(\frac{\partial O_2}{\partial K_2}\right)^2 \sigma_{K_2}^2 + \ldots$$

$$\sigma_{I_4}^2 = \sigma_{O_3}^2 = \left(\frac{\partial O_3}{\partial I_3}\right)^2 \sigma_{I_3}^2 + \left(\frac{\partial O_3}{\partial I_{M_3}}\right)^2 \sigma_{I_{M_3}}^2 + \left(\frac{\partial O_3}{\partial I_{I_3}}\right)^2 \sigma_{I_{I_3}}^2 + \left(\frac{\partial O_3}{\partial K_3}\right)^2 \sigma_{K_3}^2 + \ldots$$

and so on for as many elements as are in the system [Bentley 1983].

Some components have errors specified by an error band; instead of a gaussian distribution of errors the error is specified as a rectangular distribution with width $2h$ centered on the ideal response. The variance of a rectangular distribution is $h^2/3$. If a number of these rectangular distributions are combined through multiplication the result approximates a gaussian distribution (by the Central Limit Theorem). If the result of combining a number of rectangular distributions approximates a gaussian distribution, then combining one more rectangular distribution will give a result that approximates a gaussian distribution. If a system contains elements with both kinds of distributions, the resulting distribution approximates a gaussian distribution.

For ideal systems that include components with error bands,

$$O = KI + a + n(k)$$

where $n(k)$ is noise with values uniformly distributed over a range $\pm h$. The output variance is calculated using

$$\sigma_O^2 = K^2\sigma_I^2 + \frac{h^2}{3}.$$

3.2 Analysis of the Subsystems

A model for each subsystem, corresponding to a block in the functional block diagram, is described in the following subsections. For each, the transfer function, additive noise, and parameter variation are given.

3.2.1 The Growing Tank

When nutrients are added to the growing tank they take a few minutes to mix thoroughly. The nutrients added accumulate in the tank, so the concentration of nutrients in the tank is the sum of all the replenishment additions up to the most recent analysis, i.e.

$$q_t(k) = K_t \sum_{i=0}^{k-1} I_t(i) + C_0 + N\left(I_t(k)\right) + n_{K_t}(k) \sum_{i=0}^{k-1} I_t(i) + K_t \sum_{i=0}^{k-1} n_t(i)$$

Where

$q_t(k)$= The concentration of a nutrient ion in moles per liter.

K_t= Inverse of the volume of the tank ($1/V_T$).

$I_t(i)$= Moles of replenishment ion added to the tank at time i.

C_0= The initial concentration of nutrients in the tank.

$N\left(I_t(k)\right)$= Nonlinear effects of buffering and complexing.

$n_{K_t}(k)$= Variation of the parameter K_t.

$n_t(k)$= Additive noise.

Taking a Z-transform changes the summation into a product, giving (leaving aside noise terms for now),

$$Q_t(z) = G_t(z) I_t(z).$$

A table of Z-transforms gives

$$G_t(z) = \frac{z^{-1} K_t}{1 - z^{-1}}.$$

as the Z-transform of a summation with a time delay.

Uptake by microorganisms introduces additive noise in the tank. Algae and bacteria grow in the tank and tubing, but their uptake is assumed to be negligible. If the nutrient solution is kept dark, algae do not grow. Bacteria grow in the peristaltic pump tubes, but this is not a problem if the tubes are changed weekly. Both algae and bacteria grow slowly in very dilute nutrient solutions.

Volatilization of ammonia introduces additive noise in controlling the ammonium concentration and measuring ammonium uptake. The amount of volatilization depends on the pH of the solution. It can be reduced by sealing the growing tank or characterized for each set of experimental conditions before long-term uptake measurements of ammonium are made. I have carried a term for $n_t(k)$, the additive noise, so the relative importance of this source of error may be determined.

The tank volume is adjusted by a set point sensor and varies less than one liter ($\pm$ 0.5 l). Variations in the volume of solution in the tank, denoted by $n_{V_T}(k)$, cause variations in the parameter K_t. The variation is denoted $n_{K_t}(k)$, with the Z-transform

$$N_{K_t}(z) \approx \left(\frac{\partial K_t}{\partial V_t}\right) N_{V_T}(z)$$

where

$$\frac{\partial K_t}{\partial V_T} = \frac{-1}{V_T^2}$$

The solution level in the growing tank decreases slowly until it drops below the conductivity sensor. Then the level is rapidly replenished, so the variation of tank volume is a sawtooth wave. A unit amplitude sawtooth wave, $s_t(k)$, has a Z-Transform

$$S_t(z) = \frac{z^{-T_t}(1-z^{-1}) + T_t^{-1} z^{-1}(1-z^{-T_t})}{(1-z^{-1})^2(1-z^{-T_t})}$$

where T_t is the period of the sawtooth wave. The root mean squared value of a sawtooth wave is $\frac{1}{2\sqrt{3}}$ if the period, T_t, is long. Multiplying the unit amplitude sawtooth by the amplitude of the volume variation, 1.0 l, gives

$$N_{V_T}(z) = S_t(z)$$

The tank volume varies $\pm$ 0.5 l, so $\sigma_{V_T} = \frac{1}{2\sqrt{3}}$. Multiplying by the partial derivative of K_t with respect to V_T gives

$$N_{K_t}(z) \approx \frac{-N_{V_T}(z)}{V_T^2}$$

and therefore

$$\sigma_{K_t} = \frac{\sigma_{V_T}}{{V_T}^2}$$

so the root mean square value of the variation in the parameter K_t is

$$\sigma_{K_t} = \frac{1}{2\sqrt{3}{V_T}^2}.$$

3.2.2 Measuring Nutrient Concentrations

Figure 3.2 shows the processes involved in measuring the concentration of nutrients. The reference solutions and the nutrient solution pass through the sensing system. The sensing system produces an output voltage for each solution. The voltages corresponding to the reference solutions are used to calculate a calibration curve for the sensing system.

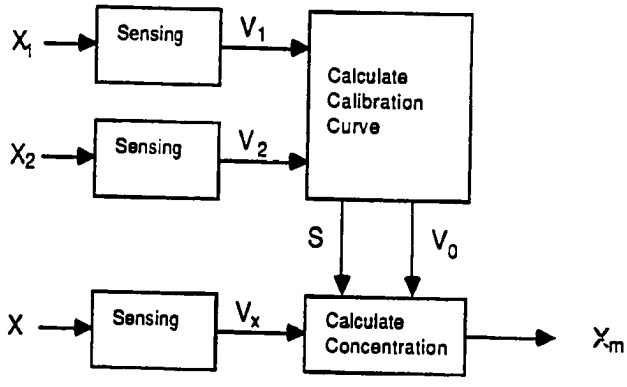

Figure 3.2 Block diagram of the measurement subsystem.

Subsections 3.2.2.1 and 3.2.2.2 describe the sensing subsystem and the calculation of the concentration, respectively.

3.2.2.1 The Sensing Subsystem

A block diagram of the sensing system is shown in figure 3.3. The tank and reference solutions are inputs to the sensing system, and voltages from ion specific electrodes are outputs. Several subsections describe the various subsystems of the sensing system. Each time the sensing system is used, it is calibrated. It is assumed that sources of proportional error except the mixing error do not change during a calibration cycle.

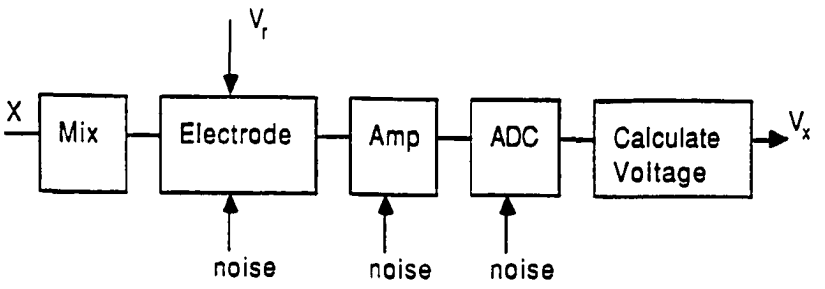

Figure 3.3 Block diagram of the sensing subsystem.

The sensing system produces steady-state outputs quickly relative to the sampling rate, so the Z-Transforms of the system are modeled as proportional constants.

3.2.2.1.1 Mixing the Solutions

The tank or reference solution must be mixed with ionic strength adjuster (ISA) before the electrode measures the concentration. Variations in pumping rate may change the

concentration of the solution. The mean pumping rate, K_{pump}, is 4.5 ml/min with a standard deviation, $\sigma_{K_{pump}}$, of 0.12 (ml/min). The concentration of an ion in the solution after mixing is

$$O_{mix} = K_{mix}I_{mix} + a + N(I_{mix}) + n_{K_{mix}}(k)I_{mix}$$

where

K_{mix}= The mixing ratio.

I_{mix}= Concentration of the input solution.

a= Concentration of the nutrient ion in the ISA (Normally zero.)

$N(I_{mix})$= Nonlinear effects on concentration when solutions are mixed.

$n_{K_{mix}}(k)$= Noise in the parameter K_{mix}.

The constant a is included for consistency with the model of section 3.1.3. The value of a is zero; no nitrate, potassium, or ammonium is in the ISA, and pH is not measured in the presence of the ISA. Nonlinear effects may occur because of complexing or buffering of ions. The mixing ratio and modifying gain are found below.

$$K_{mix} = \frac{K_{pump_{Tank}}}{K_{pump_{Tank}} + K_{pump_{ISA}}}$$

Ideally, $K_{pump_{Tank}} = K_{pump_{ISA}} = K_{pump}$, so

$$K_{mix} = 0.5$$

The sensitivity of K_{mix} to changes in pumping rate is:

$$\frac{\partial K_{mix}}{\partial K_{pump_{Tank}}} = \frac{(K_{pump_{Tank}} + K_{pump_{ISA}}) - K_{pump_{Tank}}}{(K_{pump_{Tank}} + K_{pump_{ISA}})^2}$$

$$\frac{\partial K_{mix}}{\partial K_{pump_{Tank}}} = \frac{K_{pump_{ISA}}}{(K_{pump_{Tank}} + K_{pump_{ISA}})^2}$$

$$\frac{\partial K_{mix}}{\partial K_{pump_{ISA}}} = \frac{-K_{pump_{Tank}}}{(K_{pump_{Tank}} + K_{pump_{ISA}})^2}$$

The variance of K_{mix} may be found using the sensitivities and the variance of the pumping rate:

$$\sigma^2_{K_{mix}} = \left(\frac{\partial K_{mix}}{\partial K_{pump_{ISA}}}\right)^2 \sigma^2_{K_{pump_{ISA}}} + \left(\frac{\partial K_{mix}}{\partial K_{pump_{Tank}}}\right)^2 \sigma^2_{K_{pump_{Tank}}}$$

$$K_{pump_{Tank}} = K_{pump_{ISA}} = K_{pump}$$

$$\sigma^2_{K_{pump_{Tank}}} = \sigma^2_{K_{pump_{ISA}}} = \sigma^2_{K_{pump}} = 0.0144\frac{\text{ml}^2}{\text{min}^2}$$

$$\sigma^2_{K_{mix}} = 2\left(\frac{\partial K_{mix}}{\partial K_{pump}}\right)^2 \sigma^2_{K_{pump}}$$

$$\sigma^2_{K_{mix}} = 8.9 \times 10^{-5}$$

3.2.2.1.2 The Response of Electrodes to the Solutions

The ion-specific electrodes produce outputs proportional to the logarithm of the ion concentration, x, to which they are specific.

$$V_{Elect} = S\log[K_{mix}x] + V_r + n_{Elect} + K_{T_{Elect}}\Delta T S\log[K_{mix}x] + n_{Chemical} + n_{Electrical}$$

Where

V_{Elect}= Output voltage from the electrode (mV).

S= Slope of electrode response (mV).

V_r= Reference potential for electrode pair (mV).

n_{Elect}= Repeatability of electrode output specified by manufacturer.

$K_{T_{Elect}}$= Manufacturer specified temperature coefficient (mV/ °C).

ΔT= Temperature change during a calibration cycle (°C).

$n_{Chemical}$= Effect of interfering ions (mV).

$n_{Electrical}$= Electromagnetically induced noise (mV).

The electrode response can be approximated by a straight line when the difference between the expected output voltage and the actual output voltage is small. The first order Taylor-series expansion of the logarithm function about a point y_0 is

$$\log(y) \approx \log(y_0) + \frac{d}{dy}(\log y)(y - y_0).$$

A straight line approximation to the log term of the electrode response near the target concentration, x_0, is

$$\log(K_{mix}x) \approx \log(K_{mix}x_0) + \frac{K_{mix}}{x_0 K_{mix} \ln(10)}(x - x_0)$$

where x_0 and x are the nominal and actual concentrations of the nutrient solution.

The electrode output, in millivolts, is

$$V_{Elect} \approx K_{Elect}x \ + \ \text{constants and noise.}$$

where

$$K_{Elect} = \frac{S}{x_0 \ln 10}.$$

Note that because of the logarithmic response of the electrodes, K_{mix} is part of the constants and noise.

Electrical interference at 60 Hz is filtered out effectively by the anti-aliasing amplifiers so it is ignored. (The filter gain at 60 Hz is -42.6 dB. Using a digital filter would require sampling at over 120 Hz to eliminate 60 Hz noise. It is much easier to use a simple filter than write assembly language subroutines to maintain this sample rate.) Chemical interference must be eliminated by using proper analytical techniques, as described in chapter 2.

The electrode manufacturer specifies electrode performance as an error band varying $\pm$ 2% ($\pm$ 0.40 mV) of the concentration of the solution. The electrodes also vary 2% per °C.

Assuming the electrode temperature is constant during the calibration and measurement, the additive noise is:

$$\sigma^2_{Elect} = h^2_{Elect}/3$$

$$\sigma^2_{Elect} = 0.0525 \text{ mV}^2$$

The electrodes are calibrated at every measurement cycle, so their slope, S, is known. Errors introduced in calibration are found in appendix A.

3.2.2.1.3 Amplifying the Electrode Output

The steady-state output from the electrode amplifiers is

$$V_{Amp} = G_{Amp}V_{Elect} + V_{offset} + K_{T_{Amp}}\Delta T(V_{Elect})$$

Where

$G_{Amp} = \frac{K_{Amp}}{(1+j\omega/3.33)}$

$K_{Amp} = 10$

V_{offset}= Doesn't matter if it is constant.

$K_{T_{Amp}}$= Temperature induced change in K_{Amp}.

The additive error introduced through this stage of the sensing system is

$$\sigma^2_{V_{Amp}} = K^2_{Amp}\sigma^2_{Elect} + \sigma^2_{Amp}$$

$$\sigma^2_{Amp} = 0.33 \text{ mV}^2$$

The source of variation is almost entirely temperature induced drift due to leakage current through the gate-protective diodes in the input circuit of the CA3130 operational amplifier. The input leakage current causes a voltage drop across the internal impedance of the electrode. For an electrode with a 200 megohm impedance and a temperature drift

of 10 °C, the output voltage drift for a typical amplifier would be 10 mV, but could be as large as 100 mV. Assuming a typical amplifier and a variance of 0.33 $(°C)^2$, ($\pm$ 1 °C) of the amplifier temperature during the seven minutes that is required to calibrate the electrodes, the output voltage variance would be 0.33 mV^2.

3.2.2.1.4 Analog to Digital Conversion

The analog to digital converter (ADC) may be modeled by

$$O_{ADC} = K_{ADC}V_{Amp} + a + n_{ADC}.$$

where

O_{ADC}=an integer number

K_{ADC}=0.2048 divisions per millivolt

a= a constant

n_{ADC}= Analog to digital conversion error, $\pm$0.5 divisions

The variance introduced into O_{ADC} by the error in conversion is

$$\sigma^2_{ADC} = h^2_{ADC}/3$$

giving a variance at the output of the ADC of

$$\sigma^2_{O_{ADC}} = K^2_{ADC}\sigma^2_{V_{Amp}} + \sigma^2_{ADC}$$

$$\sigma^2_{O_{ADC}} = K^2_{ADC}(K^2_{Amp}\sigma^2_{Elect} + \sigma^2_{Amp}) + \sigma^2_{ADC}.$$

The ADC may have a slight offset, but it is constant and therefore doesn't affect the calibration of the electrodes.

3.2.2.1.5 Calculating the Output Voltage

The software divides the output of the Analog to Digital converter by 2.048 to find the electrode output in millivolts. (The amplifier gain was 10 and the ADC had 0.2048 divisions per millivolt.) This result is used to calculate the concentration. The single precision number used to represent the voltage can perfectly represent the twelve bit output of the ADC. Round-off error in the division introduces an insignificant error of 1×10^{-5} % into K_V.

$$V = K_V O_{ADC}$$

$$K_V = \left(\frac{1}{2.048}\right)\left(\frac{\text{mV}}{\text{ADC division}}\right)$$

The output noise variance that is introduced in measuring the concentration of the solution is

$$\sigma_{V_x}^2 = K_V^2(K_{ADC}^2 K_{Amp}^2 K_{Elect}^2 \sigma_x^2 + K_{ADC}^2 K_{Amp}^2 \sigma_{Elect}^2 + K_{ADC}^2 \sigma_{Amp} + \sigma_{ADC}^2)$$

The noise variance introduced in the sensing process from additive sources is

$$\sigma_V^2 = K_V^2(K_{ADC}^2 K_{Amp}^2 \sigma_{Elect}^2 + K_{ADC}^2 \sigma_{Amp} + \sigma_{ADC}^2)$$

Using the previously calculated noise variance from each source, the introduced noise variance is

$$\sigma_V^2 = 0.053 + 0.0033 + 0.020 \text{ mV}^2$$

$$\sigma_V^2 = 0.077 \text{ mV}^2$$

The majority of the variance, 0.053 mV^2, is a result of uncertainty in the electrode output.

Combining the results of sections 3.2.2.1.1-3.2.2.1.5 yields a model of the sensing subsystem where

$$V_x = K_s x + V_{offset}$$

$$K_s = K_V K_{ADC} K_{Amp} K_{Elect} = \frac{S}{x_0 \ln 10}$$

where

V_x = Output voltage resulting from the concentration x (mV).

x = Concentration of nutrient ion in the culture solution (M).

V_{offset} = Offset voltage (mV).

$S \approx 56$ mV.

x_0 = Desired concentration of a nutrient ion (M).

The additive and proportional noise variance introduced is

$$\sigma_V^2 = 0.77 \text{ mV}^2$$

$$\sigma_{K_s}^2 = 8.9 \times 10^{-5}$$

3.2.2.2 Calculating Concentrations

The electrodes are calibrated using two reference solutions, one with a concentration higher than the tank concentration and one with a concentration lower than the tank concentration. The following equations are solved for S and V_0.

$$V_1 = S \log(x_1) + V_0$$

$$V_2 = S \log(x_2) + V_0.$$

Where

V_i=Measured electrode potential for reference solution i.

S=Electrode Slope (mV).

x_i=The concentration of reference solution i.

V_0=An offset potential.

The offset potential, V_0, is the sum of reference electrode potential, amplifier offset, and offset as a result of dilution when the nutrient solution is mixed with the ISA.

$$S = \frac{V_2 - V_1}{\log(x_1) - \log(x_2)}$$

$$V_0 = \frac{V_1 \log(x_2) - V_2 \log(x_1)}{\log(x_2) - \log(x_1)}$$

The output voltage from the electrode is

$$V_x = S \log(x) + V_0$$

where S and V_0 are the slope and reference potential from section 3.3.3.1. This equation can be solved for x_m, the measured concentration of nutrient ions in the culture solution,

$$x_m = 10^{\frac{V_x - V_0}{S}}.$$

In appendix A, a linear model for calculating x_m is described. The transfer function is a constant, $G_c(z) = 1$. Choosing the concentrations of the reference solutions so that $x_0 = \sqrt{x_1 x_2}$ minimizes the output variance of the measured concentration. This value, derived in appendix A, is

$$\sigma^2_{x_m} = \left(\frac{x_0 \ln 10}{S}\right)^2 \left[\sigma^2_{V_x} + \left(\frac{\sigma^2_{V_2}}{\sigma^2_{V_1} + \sigma^2_{V_2}}\right)^2 \sigma^2_{V_1} + \left(\frac{\sigma^2_{V_1}}{\sigma^2_{V_1} + \sigma^2_{V_2}}\right)^2 \sigma^2_{V_2}\right] + \sigma^2_x.$$

Removing σ^2_x, the variance of the concentration itself, gives the noise introduced in measuring the concentration;

$$\sigma^2_c = \left(\frac{x_0 \ln 10}{S}\right)^2 \left[\sigma^2_{V_x} + \left(\frac{\sigma^2_{V_2}}{\sigma^2_{V_1} + \sigma^2_{V_2}}\right)^2 \sigma^2_{V_1} + \left(\frac{\sigma^2_{V_1}}{\sigma^2_{V_1} + \sigma^2_{V_2}}\right)^2 \sigma^2_{V_2}\right].$$

Using the values from previous sections,

$$\sigma^2_c = (0.014 x_0)^2 \left(\frac{\text{moles}}{\text{liter}}\right)^2$$

$$\sigma^2_{K_c} = 8.9 \times 10^{-5}.$$

3.2.3 Correcting Nutrient Concentrations in the Culture Solution

The number of moles of nutrient ion needed to correct the concentration error is the product of the concentration error, $e(k)$, and the volume of solution in the tank, V_T. This is equal to the product of the pumping time τ, estimated pumping rate K_{pump_0}, and estimated replenishment solution concentration c_{rep_0}.

$$e(k)V_T = \tau K_{pump_0} c_{rep_0}.$$

The dimensions of this formula, as a check, are

$$\left(\frac{\text{Moles}}{\text{l}}\right)(l) = (s)\left(\frac{l}{s}\right)\left(\frac{\text{Moles}}{\text{l}}\right)$$

$$(\text{Moles}) = (\text{Moles}).$$

Solving for t gives

$$\tau = \frac{e(k)V_T}{K_{pump_0} c_{rep_0}}.$$

If K_{r_1} is the relation between the concentration error and the time the valve needs to be open (see Figure 3.4).

$$\tau = K_{r_1} e(k)$$

$$K_{r_1} = \frac{V_T}{K_{pump_0} c_{rep}}.$$

The pumping rate relates the time the valve is open to the volume pumped.

$$K_{r_2} = K_{pump_0}$$

The concentration of the replenishment solution determines the number of moles of nutrient ion that goes into the tank.

$$K_{r_3} = c_{rep_0}$$

The output of the replenishment subsystem in moles of a nutrient ion is

$$q_r(k) = K_r e(k) + n_r(k) + e(k) n_{K_r}(k)$$

where

$K_r = \frac{V_T r c_{rep}}{K_{pump_0} c_{rep}} \approx V_T$.

$n_r(k)$= Additive error in the amount of a nutrient added.

$n_{K_r}(k)$= Error in K_r.

The output, q_r, is in moles and the input, $e(k)$, is in moles/liter, so K_r has units of liters.

Additive noise and offset in the replenishment system are introduced by stretching and shrinkage of tubing in the system as pressure is applied. Proportional noise is caused by an error in the replenishment solution concentration, inaccurate estimates of the pumping rate and changes in the pumping rate as the pump tubes lose elasticity.

Pumping rate statistics were obtained using measurements made by a system that repeatedly added and removed water from a beaker sitting on an electronic analytical balance. An RS - 232 interface between the balance and the computer allowed the computer to measure the initial and final weight of water in the beaker. Testing the pumping rate with several different sets of pump tubes gave an estimate for the error in the pumping rate, K_{pump}, $\sigma_{K_{pump}} = 0.103$ ml/min. For each set of pump tubes, the error for the amount pumped had a standard deviation, $\sigma_{pump} = 0.070$ ml (when the peristaltic pump was pulling solutions up 0.8 meters). There was also some backflow when the valves were opened, so the volume intercept of the volume pumped vs. time curve was less than zero. The error in the intercept was 0.020 ml, which results a $\sigma_{offset} = 1.1 \times 10^{-5} c_{rep}$.

The replenishment solution is changed only once or twice during an experiment so the error introduced in preparation of the replenishment solution is a constant. It is assumed that this error is uniformly distributed over a $\pm$ 1% range, $\sigma^2_{c_{rep}} = (0.01 c_{rep})^2/3$, so $\sigma_{c_{rep}} =$

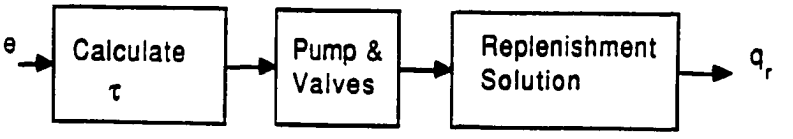

Figure 3.4 Block diagram of the replenishment subsystem.

$0.0058c_{rep}$. The pumping rate is assumed to vary as a saw-tooth wave with a period of one week, because the tubes are changed once a week as they gradually lose some of their elasticity. (The period of the change in pumping rate, T_r, is about 200.)

Referring the error in volume pumped to the output of the replenishment block gives the error in moles of nutrient ion,

$$N_r(z) = K_{r_3} N_{pump}(z)$$

$$\sigma_r(z) = K_{r_3}(\sigma_{pump} + \sigma_{offset}$$

$$\sigma_r(z) = \frac{(c_{rep} \text{ moles/liter})(0.07 \text{ ml})}{1000 \text{ ml/l}} + 1.1 \times 10^{-5} c_{rep}$$

$$\sigma_r(z) = 0.00071 c_{rep} \text{ moles}$$

for additive noise. For proportional noise,

$$N_{K_{pump}} = \sqrt{3}\sigma_{K_{pump}} S_r(z)$$

$$N_{K_{pump}}(z) \approx \frac{\partial K_r}{\partial \tau} N_{K_{pump}}(z) + \frac{\partial K_r}{\partial c_{rep}} N_{c_{rep}}(z)$$

$$N_{K_r}(z) \approx \frac{V_T}{K_{pump_0}} N_{K_{pump}}(z) + \frac{V_T}{c_{rep}} N_{c_{rep}}(z)$$

$$\sigma_{K_r}^2 = \left(\frac{V_T}{K_{pump_0}}\sigma_{K_{pump}}\right)^2 + \left(\frac{V_T}{c_{rep}}\sigma_{c_{rep}}\right)^2$$

$$\sigma_{K_r} = 0.57$$

(Assuming V_T=25 l.) Where $S_r(z)$ is the Z-Transform of a sawtooth wave with period T_r, the time between pump tube changes.

3.2.4 Measuring the Uptake

The volume of solution added to the tank is measured by finding the difference between the time when a replenishment valve was opened and the time when it was closed. The additive and proportional errors introduced are similar to the errors in the replenishment process.

$$q_u(k) = \sum_{i=0}^{k} (K_u q_r(i) + n_{K_u}(i) q_r(i) + a_u + n_u(i) + n_{a_u}(i))$$

where

$q_u(k)$= Moles of a nutrient ion used.

$K_u = \frac{V_T r c_{rep}}{V_T K_{pump_0} c_{rep_0}} \approx 1.$

$q_r(i)$= Number of moles of a nutrient added at time i.

$n_{K_u}(i)$= Noise due to error in the estimate of pumping rate.

a_u= Offset in the number of moles added.

$n_u(i)$= Noise due to additive error in the estimate of volume pumped.

$n_{a_u}(i)$= Noise due to estimate of offset in volume pumped.

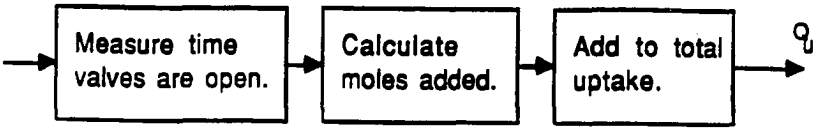

Figure 3.5 Block diagram of the uptake measurement subsystem.

The noise term n_{K_u} is found by taking the partials of K_u with respect to the pumping rate and the concentration of the replenishment solution.

$$n_{K_u} = \frac{\partial K_u}{\partial K_{pump}} n_{K_{pump}} + \frac{\partial K_u}{\partial c_{rep}} n_{c_{rep}}.$$

If $c_{rep} = c_{rep_0}$ and $K_{pump} = K_{pump_0}$,

$$n_{K_u} = \frac{n_{K_{pump}}}{K_{pump_0}} + \frac{n_{c_{rep}}}{c_{rep_0}}.$$

The variance of proportional noise,

$$\sigma^2_{K_u} = \left(\frac{\sigma_{K_{pump}}}{K_{pump_0}}\right)^2 + \left(\frac{\sigma_{c_{rep}}}{c_{rep_0}}\right)^2.$$

$$\sigma_{K_u} = 0.023.$$

The noise term n_u is due to random variation in the volume pumped. It is

$$n_u = c_{rep} n_{pump},$$

and the corresponding variance is

$$\sigma_u = \frac{0.07ml}{1000\frac{ml}{l}} c_{rep}.$$

The noise due to offset in the volume pumped is additive, but it is not random. From tests of pumped volume vs. time, $\sigma_{a_u} = 1.13 \times 10^{-5} c_{rep}$ moles.

3.3 The Control System

The Nutrient Flow System is designed to track uptake of nutrients by plants and regulate the concentration of the nutrient solution. To emphasize the dual function, Figures 3.6 and 3.7 show how the NFS block diagram may be configured as a conventional tracking system or regulator. This analysis calculates the measured concentration, $q_c(k)$, the actual concentration, $q_t(k)$, and the measured uptake, $q_u(k)$.

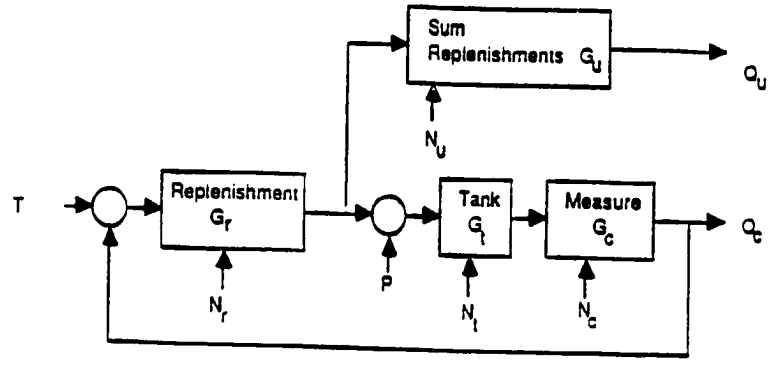

Figure 3.6 Block diagram of the NFS as a set point regulator.

Using figure 3.7, the system transfer function may be found. The functions of z have been abbreviated to improve readability, e.g. $Q_c = Q_c(z)$. The measured concentration, including additive noise is:

$$Q_c = G_c G_t (P - Q_c G_r + G_r T) + G_c G_t N_r + G_c N_t + N_c$$

$$Q_c + Q_c G_c G_t G_r = G_c G_t P + G_c G_t G_r T + G_c G_t N_r + G_c N_t + N_c$$

$$Q_c = \frac{G_c G_t P + G_c G_t G_r T}{1 + G_r G_c G_t} + \frac{G_c G_t N_r + G_c N_t + N_c}{1 + G_r G_c G_t}.$$

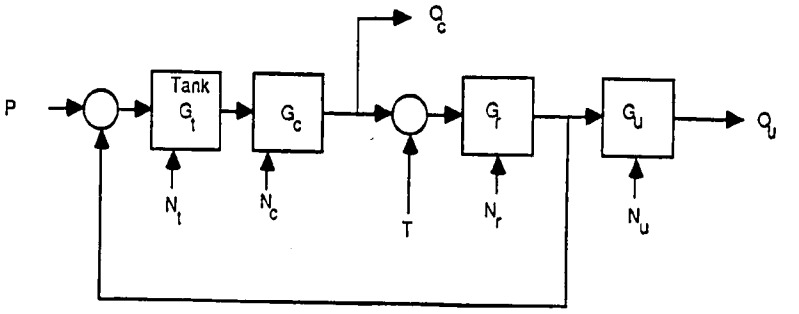

Figure 3.7 Block diagram of the NFS as a tracking system with noise sources.

The actual concentration of the tank is, including additive noise,

$$Q_t = G_t(P - Q_t G_c G_r - (-TG_r) - G_r N_c - N_r) + N_t$$

$$Q_t = G_t P - G_t G_c G_r Q_t + G_r G_t T - G_t G_r N_c - G_t N_r + N_t$$

$$Q_t(1 + G_t G_c G_r) = G_t P + G_r G_t T - G_t G_r N_c - G_t N_r + N_t$$

$$Q_t = \frac{G_t P + G_r G_t T}{1 + G_t G_c G_r} + \frac{-G_t G_r N_c - G_t N_r + N_t}{1 + G_t G_c G_r}.$$

The measured uptake is

$$Q_u = G_u Q_r.$$

Where Q_r is the output from the replenishment subsystem. The replenishment subsystem output.

$$Q_r = G_r G_c G_t(P - Q_r) - G_r T + G_r G_c N_t + G_r N_c + N_r.$$

Putting terms including Q_r on the left yields

$$Q_r + G_r G_c G_t Q_r = G_r G_c G_t P - G_r T + G_r G_c N_t + G_r N_c + N_r.$$

Factoring out the replenishment output and dividing isolates Q_r,

$$Q_r = \frac{G_r G_c G_t P - G_r T}{1 + G_r G_c G_t} + \frac{G_r G_c N_t + G_r N_c + N_r}{1 + G_r G_c G_t}.$$

Multiplying both sides by the transfer function for the uptake subsystem and adding the uptake measurement noise,

$$Q_u = \frac{G_u G_r G_c G_t P - G_u G_r T}{1 + G_r G_c G_t} + \frac{G_u G_r G_c N_t + G_u G_r N_c + G_u N_r}{1 + G_r G_c G_t} + N_{a_u} + N_u.$$

Replacing G_t, G_c, G_r, and G_u with the models found in section 3.2 gives Q_c, Q_t, Q_r, and Q_u as functions of the input signals and z with parameters K_t, K_c, K_r, and K_u. Showing functions of z explicitly to avoid confusion with parameters, the transfer functions from section 3.2 are:

$$G_t(z) = \frac{K_t z^{-1}}{1 - z^{-1}}$$

$$G_c(z) \approx K_c \quad \text{(for low frequencies.)}$$

$$G_r(z) = K_r$$

$$G_u(z) = \frac{K_u}{1 - z^{-1}}.$$

These may be used to find the measured concentration. Ignoring noise for now,

$$Q_c(z) = \frac{\frac{z^{-1}K_cK_t}{1-z^{-1}}P(z) + \frac{z^{-1}K_cK_rK_t}{1-z^{-1}}T(z)}{1 + \frac{z^{-1}K_cK_rK_t}{1-z^{-1}}}$$

$$Q_c(z) = \frac{z^{-1}K_cK_tP(z) + z^{-1}K_cK_rK_tT(z)}{(1 - z^{-1}) + z^{-1}K_cK_rK_t}$$

$$Q_c(z) = \frac{z^{-1}K_cK_tP(z) + z^{-1}K_cK_rK_tT(z)}{1 - (1 - K_cK_rK_t)z^{-1}}.$$

The actual concentration, including noise, is

$$Q_t(z) = \frac{\left(\frac{z^{-1}K_t}{1-z^{-1}}\right)P(z) + \left(\frac{z^{-1}K_t}{1-z^{-1}}\right)K_rT(z) - \left(\frac{z^{-1}K_t}{1-z^{-1}}\right)K_rN_c(z) - \left(\frac{z^{-1}K_t}{1-z^{-1}}\right)N_r(z) + N_t(z)}{1 + \frac{z^{-1}K_cK_rK_t}{1-z^{-1}}}$$

$$Q_t(z) = \frac{z^{-1}K_tP(z) + z^{-1}K_tK_rT(z) - z^{-1}K_tK_rN_c(z) - z^{-1}K_tN_r(z) + (1 - z^{-1})N_t(z)}{1 - (1 - K_cK_rK_t)z^{-1}}.$$

The measured uptake is

$$Q_u(z) = \frac{\left(\frac{K_u}{1-z^{-1}}\right) K_r \left(\frac{K_t z^{-1}}{1-z^{-1}}\right) K_c P(z) - \left(\frac{K_u}{1-z^{-1}}\right) K_r T(z)}{1 + \frac{z^{-1} K_c K_r K_t}{1-z^{-1}}}$$

$$Q_u(z) = \frac{\left(\frac{z^{-1}}{1-z^{-1}}\right) K_u K_r K_t K_c P(z) - K_u K_r T(z)}{1 - z^{-1} + z^{-1} K_c K_r K_t}$$

$$Q_u(z) = \frac{\left(\frac{z}{1-z^{-1}}\right) K_u K_r K_t K_c P(z) - K_u K_r T(z)}{1 - (1 - K_c K_r K_t) z^{-1}}.$$

If $K_u = 1$, $K_c = 1$, $K_r = V_T$, and $K_t = \frac{1}{V_T}$, (for deadbeat control), the transfer functions are simpler. For now ignoring noise in the system,

$$Q_c(z) = z^{-1} K_t P(z) + z^{-1} T(z)$$

$$Q_u(z) = \frac{z^{-1} P(z)}{1 - z^{-1}} - K_r T(z).$$

The corresponding time domain results are

$$q_c(k) = K_t p(k-1) + t(k-1)$$

$$q_u(k) = -K_r t(k) + \sum_{l=0}^{k-1} p(k-1).$$

The measured concentration at any time k is the sum of the replenishment input and the plant uptake from the previous analysis at time $k-1$. The measured uptake is the sum of all additions minus the nutrients necessary to raise the concentration to the target level.

3.4 Effect of Noise and Parameter Variation

In this subsection the effects of additive noise, of multiplicative noise, and their combined effects on the measured concentration, actual concentration, and the measured uptake are found.

3.4.1 Disturbance from Additive Noise

The outputs may be divided into the nominal output and the output due to disturbances, which include noise and uptake by the plants. The disturbance part of the measured concentration is

$$Q_{c_d}(z) = \frac{G_c(z)G_t(z)P(z) + G_c(z)G_t(z)N_r(z) + G_c(z)N_t(z) + N_c(z)}{1 + G_t(z)G_c(z)G_r(z)}$$

$$Q_{c_d}(z) = \frac{\left(\frac{z^{-1}K_t}{1-z^{-1}}\right)K_cP(z) + \left(\frac{z^{-1}K_t}{1-z^{-1}}\right)K_cN_r(z) + K_cN_t(z) + N_c(z)}{1 + \frac{z^{-1}K_cK_rK_t}{1-z^{-1}}}$$

$$Q_{c_d} = \frac{z^{-1}K_cK_tP(z) + z^{-1}K_cK_tN_r(z) + (1-z^{-1})K_cN_t(z) + (1-z^{-1})N_c(z)}{1-(1-K_cK_rK_t)z^{-1}}.$$

For deadbeat control, $K_c = 1, K_t = \frac{1}{V_T}, K_r = V_T$ and

$$Q_{c_d}(z) = z^{-1}\frac{P(z)}{V_T} + z^{-1}\frac{N_r(z)}{V_T} + (1-z^{-1})N_t(z) + (1-z^{-1})N_c(z).$$

The actual concentration of nutrients in the tank is,

$$Q_t(z) = \frac{z^{-1}K_tP(z) + z^{-1}K_tK_rT(z) - z^{-1}K_tK_rN_c(z) - z^{-1}K_tN_r(z) + (1-z^{-1})N_t(z)}{1-(1-K_cK_rK_t)z^{-1}}.$$

The disturbance of the actual concentration of nutrients in the tank due to additive noise and uptake is,

$$Q_{t_d}(z) = \frac{z^{-1}K_tP(z) - z^{-1}K_tK_rN_c(z) - z^{-1}K_tN_r(z) + (1-z^{-1})N_t(z)}{1-(1-K_cK_rK_t)z^{-1}}.$$

For $K_c = 1, K_t = \frac{1}{V_T}, K_r = V_T$,

$$Q_{t_d}(z) = z^{-1}\frac{P(z)}{V_T} - z^{-1}N_c(z) - z^{-1}\frac{N_r(z)}{V_T} + (1-z^{-1})N_t(z).$$

Uptake of nutrients causes variations in concentration, so in the previous two cases it was included as a disturbance or noise input. Nutrient uptake is a signal (vs. a noise or

disturbance) in the measured uptake calculation, so unlike in the previous two cases the uptake is not included as part of the disturbance output. The error in the measured uptake due to additive noise is

$$Q_{u_d}(z) = \frac{G_u(z)G_r(z)G_c(z)N_t(z) + G_u(z)G_r(z)N_c(z) + G_u(z)N_r(z)}{1 + G_r(z)G_c(z)G_t(z)} + N_{a_u} + N_u.$$

$$Q_{u_d}(z) = \frac{\left(\frac{K_u}{1-z^{-1}}\right) K_r K_c N_t(z) + \left(\frac{K_u}{1-z^{-1}}\right) K_r N_c(z) + \left(\frac{K_u}{1-z^{-1}}\right) N_r(z)}{1 + \frac{z^{-1}K_cK_rK_t}{1-z^{-1}}} + N_{a_u}(z) + N_u(z)$$

$$Q_{u_d}(z) = \frac{K_u K_r K_c N_t(z) + K_u K_r N_c(z) + K_u N_r(z)}{1 - (1 - K_c K_r K_t)z^{-1}} + N_{a_u} + N_u(z).$$

For deadbeat control, $K_u = 1$, $K_c = 1$, $K_t = \frac{1}{V_T}$, and $K_r = V_T$, so

$$Q_{u_d}(z) = V_T N_t(z) + V_T N_c(z) + N_r(z) + N_{a_u} + N_u(z).$$

3.4.2 Sensitivity to Variations of Parameters

Sensitivity of the measured concentration to variations of parameters may be found by taking partial derivatives of the measured concentration, $Q_c(z)$, with respect to the parameters that vary; K_t, K_c, and K_r.

Using the quotient rule for differentiation, the sensitivity to variation of the volume of nutrient solution in the tank is

$$\frac{\partial Q_c(z)}{\partial K_t} = \frac{z^{-1}K_cP(z)(1-(1-K_cK_rK_t)z^{-1}) - z^{-1}K_cK_tP(z)(K_cK_rz^{-1})}{(1-(1-K_cK_rK_t)z^{-1})^2}$$

$$+\frac{z^{-1}K_rK_cT(z)(1-(1-K_cK_rK_t)z^{-1}) - z^{-1}K_cK_tT(z)(K_cK_rz^{-1})}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Expanding the terms gives

$$\frac{\partial Q_c(z)}{\partial K_t} = \frac{z^{-1}K_cP(z) - (z^{-1}K_cP(z) - z^{-1}K_tK_c^2K_rP(z))z^{-1} - z^{-2}K_tK_rK_c^2P(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}$$

$$+\frac{z^{-1}K_rK_cT(z) - (z^{-1}K_rK_cT(z) - K_tK_r^2K_c^2T(z))z^{-1} - z^{-2}K_tK_r^2K_c^2T(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Noting the cancellations yields

$$\frac{\partial Q_c(z)}{\partial K_t} = \frac{(z^{-1}-z^{-2})K_cP(z) + (z^{-1}-z^{-2})K_rK_cT(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}$$

$$\frac{\partial Q_c(z)}{\partial K_t} = \frac{(z^{-1}-z^{-2})(K_cP(z) + K_rK_cT(z))}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Similarly, the sensitivity of measured concentration to variation in the parameter K_c is

$$\frac{\partial Q_c(z)}{\partial K_c} = \frac{(z^{-1}-z^{-2})(K_tP(z) + K_tK_rT(z))}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

The sensitivity of measured concentration to changes in pumping rate is

$$\frac{\partial Q_c(z)}{\partial K_r} = \frac{z^{-1}K_tK_cP(z)}{(1-(1-K_cK_rK_t)z^{-1})^2} + \frac{(z^{-1}-z^{-2})K_tK_cT(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Now the steps are repeated for the actual concentration. The actual concentration is

$$Q_t(z) \approx \frac{z^{-1}K_tK_rT(z)}{1-(1-K_cK_rK_t)z^{-1}}.$$

Using the quotient rule for differentiation gives the sensitivity to changes in the volume of nutrient solution.

$$\frac{\partial Q_t(z)}{\partial K_t} \approx \frac{(z^{-1}K_rT(z))(1-z^{-1}+K_cK_rK_tz^{-1}) - (z^{-1}K_rK_tT(z))(K_cK_tz^{-1})}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Expanding the terms gives

$$\frac{\partial Q_t(z)}{\partial K_t} \approx \frac{z^{-1}K_rT - z^{-2}K_rT(z) + z^{-2}K_cK_r^2K_tT(z) - z^{-2}K_cK_r^2K_tT(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Noting the canceling terms gives an expression for the sensitivity of the actual concentration to changes in the volume of nutrient solution.

$$\frac{\partial Q_t(z)}{\partial K_t} \approx \frac{(z^{-1}-z^{-2})K_r T(z)}{(1-(1-K_c K_r K_t)z^{-1})^2}.$$

Similarly, the sensitivity of actual tank concentration to variation in the replenishment rate, K_r, is

$$\frac{\partial Q_t(z)}{\partial K_r} \approx \frac{(z^{-1}-z^{-2})K_t T(z)}{(1-(1-K_c K_r K_t)z^{-1})^2}.$$

The sensitivity of the actual concentration to multiplicative error in the measurement of concentration is

$$\frac{\partial Q_t(z)}{\partial K_c} \approx \frac{-(z^{-1}K_t K_r T(z))(K_r K_t z^{-1})}{(1-(1-K_c K_r K_t)z^{-1})^2}$$

$$\frac{\partial Q_t(z)}{\partial K_c} \approx \frac{-z^{-2}K_t^2 K_r^2 T(z)}{(1-(1-K_c K_r K_t)z^{-1})^2}.$$

Sensitivities of uptake measurements to variations of parameters are partial derivatives of $Q_u(z)$ with respect to K_u as well as K_t, K_c, K_r.

$$Q_u(z) = \frac{\left(\frac{z^{-1}}{1-z^{-1}}\right) K_u K_r K_t K_c P(z) - z^{-1} K_u K_r T(z)}{1-(1-K_c K_r K_t)z^{-1}}.$$

Grouping the terms for clarification,

$$Q_u(z) = \frac{\left(\frac{z^{-1}K_u K_r K_c P(z)}{1-z^{-1}}\right) K_t - z^{-1} K_u K_r T(z)}{1-(1-K_c K_r K_t)z^{-1}}.$$

Applying the quotient rule for differentiation gives

$$\frac{\partial Q_u(z)}{\partial K_t} = \frac{\left(\frac{z^{-1}K_uK_rK_cP(z)}{1-z^{-1}}\right)\left[(1-(1-K_cK_rK_t)z^{-1}) - K_t(K_cK_rz^{-1})\right]}{(1-(1-K_cK_rK_t)z^{-1})^2}$$

$$-\frac{(-z^{-1}K_rT(z))(K_uK_cK_rz^{-1})}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Expanding the terms,

$$\frac{\partial Q_u(z)}{\partial K_t} = \frac{\left(\frac{z^{-1}K_uK_rK_cP(z)}{1-z^{-1}}\right)\left[1-z^{-1}+K_cK_rK_tz^{-1}-K_tK_cK_rz^{-1}\right]+z^{-2}K_cK_uK_r^2T(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Eliminating canceling terms results in

$$\frac{\partial Q_u(z)}{\partial K_t} = \frac{\left(\frac{z^{-1}K_uK_rK_cP(z)}{1-z^{-1}}\right)\left[1-z^{-1}\right]+z^{-2}K_cK_uK_r^2T(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Finally, the sensitivity of measured uptake to variations in the volume of nutrient solution in the tank is,

$$\frac{\partial Q_u(z)}{\partial K_t} = \frac{z^{-1}K_uK_rK_cP(z)+z^{-2}K_cK_uK_r^2T(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Similarly, the sensitivity to multiplicative errors when measuring concentration is,

$$\frac{\partial Q_u(z)}{\partial K_c} = \frac{z^{-1}K_uK_rK_tP(z)+z^{-2}K_tK_uK_r^2T(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

and the sensitivity of measured uptake to replenishment rate is, differentiating the first term as before,

$$\frac{\partial Q_u(z)}{\partial K_r} = \frac{z^{-1}K_uK_cK_tP(z)}{(1-(1-K_cK_rK_t)z^{-1})^2} + \frac{\partial}{\partial K_r}\left(\frac{-z^{-1}K_uK_rT(z)}{1-(1-K_cK_rK_t)z^{-1}}\right).$$

Applying the quotient rule to the second term,

$$\frac{\partial Q_u(z)}{\partial K_r} = \frac{z^{-1}K_uK_cK_tP(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}$$

$$+\frac{-z^{-1}K_uT(z)(1-(1-K_cK_rK_t)z^{-1}) - (-z^{-1}K_uK_rT(z))(K_cK_tz^{-1})}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Multiplying the factors above together yields

$$\frac{\partial Q_u(z)}{\partial K_r} = \frac{z^{-1}K_uK_cK_tP(z) + -z^{-1}K_uT(z) + z^{-2}K_uT(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}$$

$$+\frac{-z^{-2}K_uK_rK_cK_tT(z) + z^{-2}K_uK_rK_cK_tT(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

Noting the canceling terms gives an expression for the sensitivity of the measured uptake to multiplicative noise in the replenishment subsystem

$$\frac{\partial Q_u(z)}{\partial K_r} = \frac{z^{-1}K_uK_cK_tP(z) + (-z^{-1}+z^{-2})K_uT(z)}{(1-(1-K_cK_rK_t)z^{-1})^2}.$$

The sensitivity to errors in the estimate of the replenishment rate is

$$\frac{\partial Q_u(z)}{\partial K_u} = \frac{\frac{z^{-1}K_rK_tK_cP(z)}{1-z^{-1}} - z^{-1}K_rT(z)}{1-(1-K_cK_rK_t)z^{-1}}.$$

The values of K_t, K_c, K_r, and K_u needed to be shown explicitly to perform differentiation. Now the sensitivities for the three outputs can be found when the parameters are assigned their nominal values, $K_c = 1, K_t = \frac{1}{V_T}, K_r = V_T$ and $K_u = 1$.

Making use of the assumption that the plant uptake causes a small change in concentration (i.e. $\frac{P(z)}{V_T} << T(z)$), the sensitivities of the measured concentration are

$$\frac{\partial Q_c(z)}{\partial K_c} \approx (z^{-1}-z^{-2})\left(\frac{P(z)}{V_T} + T(z)\right) \approx (z^{-1}-z^{-2})T(z)$$

$$\frac{\partial Q_c(z)}{\partial K_t} \approx (z^{-1}-z^{-2})\left(\frac{P(z)}{V_T} + V_TT(z)\right) \approx (z^{-1}-z^{-2})V_TT(z)$$

$$\frac{\partial Q_c(z)}{\partial K_r} \approx \frac{-z^{-2}P(z)}{{V_T}^2} + (z^{-1}-z^{-2})\frac{T(z)}{V_T} \approx (z^{-1}-z^{-2})\frac{T(z)}{V_T}.$$

The sensitivities of the actual concentration to the changes in the parameters are:

$$\frac{\partial Q_t(z)}{\partial K_c} = -z^{-2}T(z)$$

$$\frac{\partial Q_t(z)}{\partial K_t} \approx (z^{-1} - z^{-2})\left(\frac{P(z)}{V_T} + V_T T(z)\right) \approx (z^{-1} - z^{-2}) V_T T(z)$$

$$\frac{\partial Q_t(z)}{\partial K_r} \approx \frac{-z^{-2} P(z)}{{V_T}^2} + (z^{-1} - z^{-2})\frac{T(z)}{V_T} \approx (z^{-1} - z^{-2})\frac{T(z)}{V_T}.$$

It is interesting that if K_c is a constant, the sensitivity of the measured concentration to K_c is zero, less than the sensitivity of the actual concentration to K_c (as would be expected.)

The sensitivities of the measured uptake to changes in the parameters are:

$$\frac{\partial Q_u(z)}{\partial K_t} \approx z^{-1} V_T P(z) + z^{-2} {V_T}^2 T(z) \approx z^{-2} {V_T}^2 T(z)$$

$$\frac{\partial Q_u(z)}{\partial K_c} \approx z^{-1} P(z) + z^{-2} V_T T(z) \approx z^{-2} V_T T(z)$$

$$\frac{\partial Q_u(z)}{\partial K_r} \approx \frac{z^{-1} P(z)}{V_T} + (z^{-1} - z^{-2}) T(z) \approx (z^{-1} - z^{-2}) T(z)$$

$$\frac{\partial Q_u(z)}{\partial K_u} \approx \frac{z^{-1} P(z)}{1 - z^{-1}} - z^{-1} V_T T(z).$$

Note the integrating term in the last sensitivity. While errors in the actual replenishment rate do NOT cause cumulative errors in the measured uptake, errors in the estimate of this rate do cause cumulative errors.

3.4.3 Combined Effects of Additive and Proportional Noise

Disturbances of the output resulting from both additive noise and parameter variations are, for the measured concentration

$$Q_{c_d}(z) = z^{-1}P(z)/V_T + z^{-1}N_r(z)/V_T + (1-z^{-1})N_t(z) + (1-z^{-1})N_c(z)$$

$$+\left(\frac{\partial Q_c}{\partial K_c}\right)N_{K_c}(z) + \left(\frac{\partial Q_c}{\partial K_t}\right)N_{K_t}(z) + \left(\frac{\partial Q_c}{\partial K_r}\right)N_{K_r}(z).$$

For the actual concentration, disturbances from additive noise and parameter variation are:

$$Q_{t_d}(z) = z^{-1}K_tP(z) - z^{-1}K_tK_rN_c(z) - z^{-1}K_tN_r(z) + (1-z^{-1})N_t(z)$$

$$+\left(\frac{\partial Q_t}{\partial K_c}\right)N_{K_c}(z) + \left(\frac{\partial Q_t}{\partial K_t}\right)N_{K_t}(z) + \left(\frac{\partial Q_t}{\partial K_r}\right)N_{K_r}(z).$$

For the measured uptake the disturbances are

$$Q_{u_d}(z) = V_TN_t(z) + V_TN_c(z) + N_r(z) + N_u(z) + N_{a_u}(z) + \left(\frac{\partial Q_u}{\partial K_c}\right)N_{K_c}(z) + \left(\frac{\partial Q_u}{\partial K_t}\right)N_{K_t}(z)$$

$$+\left(\frac{\partial Q_u}{\partial K_r}\right)N_{K_r}(z) + \left(\frac{\partial Q_u}{\partial K_u}\right)N_{K_u}(z).$$

Each of these results is expanded in a following subsection. First the Z-transform associated with each symbol is substituted, then the result is transformed to a discrete time expression, and finally the variance of the discrete time expression is given.

3.4.3.1 Effect on Measured Concentration

Substituting in the Z-transforms gives the effect of disturbance inputs and parameter variations on the concentration measurements. In the Z-domain,

$$Q_{c_d}(z) = \frac{z^{-1}P(z)}{V_T} + \frac{z^{-1}N_r(z)}{V_T} + (1-z^{-1})N_t(z) + (1-z^{-1})N_c(z)$$

$$+(z^{-1}-z^{-2})T(z)N_{K_c}(z) + (z^{-1}-z^{-2})V_TT(z)N_{K_t}(z)$$

$$+(z^{-1}-z^{-2})\frac{T(z)}{V_T}N_{K_r}(z),$$

The error caused by microorganisms and volitalization is in units of moles/liter. It is replaced by using

$$N_t(z) = \frac{N_p(z)/\mathrm{V_T}}{1 - z^{-1}}$$

where $N_p(z)$ is error in uptake of plants due to microorganisms and volitalization, in moles.

$$Q_{c_d}(z) = \frac{z^{-1}P(z)}{\mathrm{V_T}} + \frac{z^{-1}N_r(z)}{\mathrm{V_T}} + (1 - z^{-1})\frac{N_p(z)/\mathrm{V_T}}{1 - z^{-1}} + (1 - z^{-1})N_c(z)$$
$$+(z^{-1} - z^{-2})T(z)N_{K_c}(z) + (z^{-1} - z^{-2})\mathrm{V_T}T(z)1.73\sigma_{K_t}S_t(z)$$
$$+(z^{-1} - z^{-2})\frac{T(z)}{\mathrm{V_T}}1.73\sigma_{K_r}S_r(z).$$

$S_t(z)$ and $S_r(z)$ are sawtooth waves representing the variation of the tank volume and pumping rate. In the time domain,

$$q_{c_d}(k) = \frac{p(k-1)}{\mathrm{V_T}} + \frac{n_r(k-1)}{\mathrm{V_T}} + \frac{n_p(k)}{\mathrm{V_T}}$$
$$+n_c(k) - n_c(k-1) + t(k-1)n_{K_c}(k-1) - t(k-2)n_{K_c}(k-2)$$
$$+\mathrm{V_T}t(k-1)\left(\sum_{n=1}^{\infty}\delta(k - n\mathrm{T}_t) - \frac{1}{\mathrm{T}_t}u(k-1)\right)1.73\sigma_{K_t}$$
$$+\frac{t(k-1)}{\mathrm{V_T}}\left(\sum_{n=1}^{\infty}\delta(k - n\mathrm{T}_r) - \frac{1}{\mathrm{T}_r}u(k-1)\right)1.73\sigma_{K_r}.$$

The error term resulting from the sawtooth variation in the replenishment rate and tank volume is shown in Figure 3.8. The error is small during the ramp part of the sawtooth wave, then the sudden change as the sawtooth goes back to zero produces a much larger error.

The average squared value of the disturbance output is, assuming $t(k)$ is constant,

$$\sigma_{q_c}^2 = \left[\frac{\sigma_p}{\mathrm{V_T}} + \frac{\sigma_{n_p}}{\mathrm{V_T}}\right]^2 + [\sigma_{K_c}t - \sigma_{K_c}t]^2 + {\mathrm{V_T}}^2t^2\frac{\sigma_{K_t}^2}{\mathrm{T}_t}(1 - 1/\mathrm{T}_t) + \frac{t^2\sigma_{K_r}^2}{\mathrm{T}_r{\mathrm{V_T}}^2}(1 - 1/\mathrm{T}_r)$$
$$+\sigma_c^2 + \sigma_c^2 + \frac{\sigma_r^2}{{\mathrm{V_T}}^2}.$$

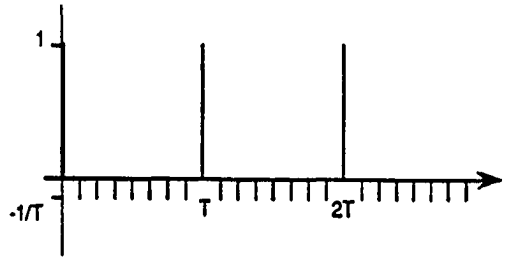

Figure 3.8 Error signal resulting from sawtooth variation in a parameter.

Noise that is correlated is included in the square brackets. Uncorrelated noise can be calculated using sum of squares because the expected values of the cross-terms are zero, but the cross-terms must be included for correlated noise. The variance of the noise introduced in the measurement subsystem, σ_c^2, appears twice because $n_c(k)$ and $n_c(k-1)$ are not correlated.

Using the nominal values of V_T, T_t, T_r, t, and the noise variances calculated earlier in the chapter gives

$$\sigma_{q_c}^2 = \frac{\alpha^2}{12}x_0^2 + (0.022)^2 x_0^2$$

where α is the hysteresis built into the control loop and x_0 is the target concentration. If $\alpha = 0$, so there is no hysteresis in the control loop, $\sigma_{q_c} = 0.044x_0$.

3.4.3.2 Effect on Actual Concentration

Substituting the Z-Transforms gives the effect of disturbance inputs and parameter variations on the actual concentration. In the Z-domain,

$$Q_{t_d} = z^{-1}P(z)\mathrm{V_T} + \frac{z^{-1}N_r(z)}{\mathrm{V_T}} + (1-z^{-1})\frac{N_p(z)/\mathrm{V_T}\sigma_t}{(1-z^{-1})} - z^{-1}N_c$$
$$+T(z)\sigma_{K_c} + (z^{-1}-z^{-2})\mathrm{V_T}T(z)S_t(z)1.73\sigma_{K_t}$$
$$+(z^{-1}-z^{-2})\frac{T(z)}{\mathrm{V_T}}S_r(z)1.73\sigma_{K_r}.$$

In the time domain,

$$q_{t_d}(k) = \frac{pu(k-1)}{\mathrm{V_T}} - \frac{n_r(k-1)}{\mathrm{V_T}} + \frac{n_p(k)}{\mathrm{V_T}} - n_c(k-1) + t(k-2)\sigma_{K_c}$$
$$+\mathrm{V_T}t(k-1)\left(\sum_{n=1}^{\infty}\delta(k-n\mathrm{T}_t) - \frac{1}{\mathrm{T}_t}u(k-1)\right)1.73\sigma_{K_t}$$
$$+\frac{t(z)}{\mathrm{V_T}}\left(\sum_{n=1}^{\infty}\delta(k-n\mathrm{T}_r) - \frac{1}{\mathrm{T}_r}u(k-1)\right)1.73\sigma_{K_r}.$$

The average squared value of the disturbance output is,

$$\sigma^2_{q_t} = \left[\frac{\sigma_p}{\mathrm{V_T}} + \frac{\sigma_{n_p}}{\mathrm{V_T}}\right]^2 + \sigma_{K_c}t + \mathrm{V_T}^2t^2\frac{\sigma^2_{K_t}}{\mathrm{T}_t}(1-1/\mathrm{T}_t) + \frac{t^2\sigma^2_{K_r}}{\mathrm{T}_r\mathrm{V_T}^2}(1-1/\mathrm{T}_r)$$
$$+\sigma^2_c + \frac{\sigma^2_r}{\mathrm{V_T}^2}.$$

Note that the variance of the noise introduced in the measurement subsystem appears once. The actual concentration is less affected by measurement error than is the measured concentration.

Using values for the noise variance calculated earlier in the chapter and simplifying gives

$$\sigma^2_{q_t} = \frac{\alpha^2}{12}x_0^2 + (0.017)^2x_0$$

where α is the hysteresis built into the control loop. If hysteresis is not allowed in the control loop ($\alpha = 0$), $\sigma_{q_t} = 0.032x_0$.

3.4.3.3 Effect on Measured Uptake

The effect of the disturbance inputs and parameter variations on the measured uptake, $Q_{u_d}(z)$ is, in the Z domain,

$$Q_{u_d}(z) = +V_T N_t(z) + V_T N_c(z) + N_r(z) + N_u(z) + N_{a_u}$$

$$-z^{-2}V_T T(z)N_{K_C}(z) - z^{-2}V_T{}^2 T(z)N_{K_t}(z) + (z^{-1} - z^{-2})T(z)N_{K_r}(z)$$

$$+\left(\frac{z^{-1}}{1-z^{-1}}P(z) - z^{-1}V_T T(z)\right)N_{K_u}(z).$$

Replacing some of the noise functions with their Z-transforms,

$$Q_{u_d}(z) = +V_T N_t(z) + V_T N_c(z) + N_r(z) + N_u(z) + N_{a_u}$$

$$-z^{-2}V_T T(z)\frac{\sigma_{K_c}}{1-z^{-1}} - z^{-2}V_T{}^2 T(z)S_t(z)1.73\sigma_{K_t}$$

$$+(z^{-1} - z^{-2})T(z)S_r(z)1.73\sigma_{K_r}$$

$$+\left(\frac{z^{-1}}{1-z^{-1}}P(z) - z^{-1}V_T T(z)\right)S_u(z)1.73\sigma_{K_u}.$$

In the time domain this is,

$$q_{u_d}(k) = \sum_{i=0}^{k} V_T n_t(i) + V_T n_c(k) + n_r(k) + \sum_{i=0}^{k} n_u(i) + \sum_{i=0}^{k} n_{a_u}(i) \; - V_T t(k-2)\sigma_{K_c}(k)$$

$$+V_t^2 t(k-2)1.73\sigma_{K_t} + t(k-2)\left(\sum_{n=1}^{k}\delta(k - nT_r) - \frac{1}{T_r}u(k-1)\right)1.73\sigma_{K_r}$$

$$+\sigma_{K_u}\sum_{n=0}^{k-1} s_u(n)p(n) - V_T t(k-1)1.73\sigma_{K_u}s_u(k).$$

The expected error of the error in uptake measurements has a variance

$$\sigma_{q_u}^2 = \left(\sum_{n=0}^{k}\sigma_t\right)^2 + V_T{}^4 t^2\sigma_{K_t}^2 + \left(t^2\frac{\sigma_{K_r}^2}{T_r}(1 - 1/T_r)\right)^{1/2}$$

$$+\sigma_{K_u}\sum_{n=0}^{k-1} p(n) + V_T t\sigma_{K_u} + V_T{}^2 t^2\sigma_{K_c}^2 + V_T{}^2\sigma_c^2 + \sigma_r^2 + \sum^{k}\sigma_u^2$$

$$+\left(\sum^{k}\sigma_{a_u}\right)^2.$$

The source of each term in the above expression for σ_{q_u} is given below. Typically, $V_T = 25$ liters, $c_{rep} = 10x_0$, and $T_r = 200$ (where x_0 is the target concentration). Each of the terms in the above equation may be expanded to give an expression that depends only on x_0 and the number of replenishments, k. (All of the error terms have units of moles^2.)

The term $(\sum_{n=0}^{k} \sigma_t)^2$ is due to uptake by microorganisms and volatilization. It is not considered because further investigation is needed to characterize it.

The term $V_T{}^4 x_0^2 \sigma_{K_t}^2$ is error due to changes in tank volume. It is $0.29^2 x_0^2$ for the nominal values of the parameters.

The term $\left(t^2 \frac{\sigma_{K_r}^2}{T_r}(1 - 1/T_r)\right)^{1/2} \approx \frac{x_0 \sigma_{K_r}}{\sqrt{T_r}}$. It is due to changes in the pumping rate of the replenishment solution, and is equal to $\frac{x_0^2 0.57^2}{\sqrt{200}} = 0.04^2 x_0^2$.

The term $\sigma_{K_u} \sum_{n=0}^{k} p(n)$ is due to error in the estimate of the pumping rate of the peristaltic pump. It is $0.023^2 q_u^2(k)$.

The term $V_T x_0 \sigma_{K_u}$ is due to error in the initial preparation of the nutrient solution. It is $25^2(0.023)^2 x_0^2 = 0.515^2 x_0^2$.

The term $V_T{}^2 \sigma_c^2$ is due to errors in the estimate of the concentration of a nutrient in the tank. It is $((25l)(0.014)x_0)^2 = (0.35x_0)^2$.

The term σ_r^2 is due to the error in the amount of nutrients added to the tank during the most recent replenishment. It is $(0.000071x_0)^2$.

The term $V_T{}^2 x_0^2 \sigma_{K_c}^2$ is due to proportional error in mixing ionic strength adjuster and nutrient solutions. It is equal to $(25(0.00148)x_0)^2$, which is $(0.037x_0)^2$ for the nominal values of parameters.

The term $\sum_{n=0}^{k} \sigma_u^2$ is due to additive noise in the volume of replenishment solution delivered by the peristaltic pump. It is $\sum c_{rep} \sigma_{pump}$, which is $\sum 0.0007^2 x_0^2$ for the nominal parameter values.

The term $(\sum_{n=0}^{k} \sigma_{a_u})^2$ is due to error in the estimate of σ_{offset}. It is $k^2(1.1 \times 10^{-4} x_0)^2$.

The total uptake, $q_u(k) = k\alpha V_T x_0$.

3.5 Summary of Analysis Results

A relative or normalized error may be defined as the error in the measured uptake divided by the total uptake. The total uptake may be expressed in terms of k, the number of replenishments to the concentration; α, the hysteresis in the control loop; and x_0, the target concentration. Dividing all the terms of the expression for σ_{q_c} by the total uptake, $V_T k\alpha x_0$, gives an expression for normalized error in terms of k and α.

$$\sigma_n^2 = 5.3 \times 10^{-4} + \frac{8.6 \times 10^{-4}}{k^2\alpha^2} + \frac{7.8 \times 10^{-10}}{k\alpha^2} + \frac{1.9 \times 10^{-11}}{\alpha^2}.$$

Figure 3.9 shows how this function varies with k and α.

A reasonable choice for α is 0.01 - 0.1, in conjunction with a $k > 50$. These values imply fairly long term measurements. Making accurate short term measurements would require much closer control of volume, pumping rate, and initial preparation of the solution as well as more accurate concentration measurements.

Tables 3.1 and 3.2 summarize the contributions of various sources to the total error in concentration when $V_T = 25, \alpha = 0.01$, $k = 100$, and $c_{rep} = 10x_0$. The only important contributions to the variance of the concentration are from the concentration measurements, the mixing ratio, and hysteresis in the control loop. The major sources of error in concentration control are electrode performance, digitization error, and mixing ratio error. They contribute 54%, 20%, and 18% of the error in concentration control, respectively. The root mean square error in concentration measurements is 2.2% of the target concentration for concentrations in the range where electrode performance is linear ($x_0 > 1 \times 10^{-5}$). For example, if the target concentration was 0.1 mM the expected root mean square deviation of the concentration measurements would be 0.0022 mM.

For this example, important errors in the uptake measurements are caused by errors in the concentration measurements (14%), the pumping rate (38%), the initial preparation of

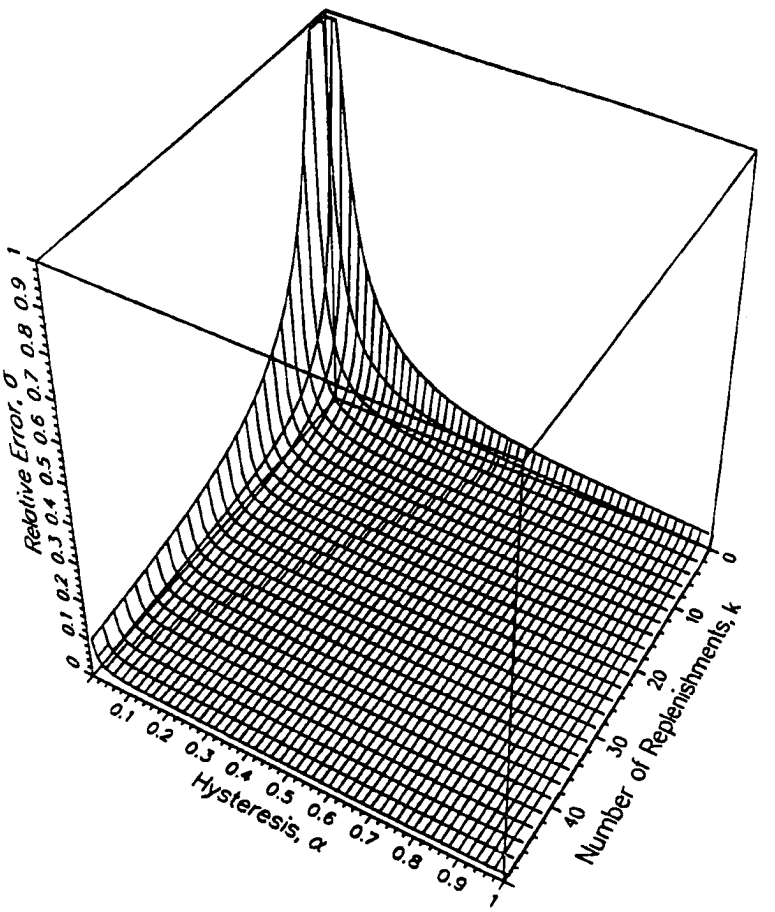

Figure 3.9 Uptake error as function of hysteresis and number of replenishments.

the nutrient solutions (38%), and the volume of nutrient solution (9%). For this example, the standard deviation of the uptake measurements is estimated to be 3.7% of the total uptake. If the target concentration was 0.1 mM, the total uptake would be $2.5 \times 10^{-3} \pm 9.3 \times 10^{-5}$ moles of nutrient ion. Figure 3.9 shows how the error in uptake measurements varies with changes in k and α.

Table 3.1 *Sources of error in measured nutrient concentrations.*

Source of error	Variance
Hysteresis	8.33×10^{-6}
Mixing Ratio	8.9×10^{-5}
Concentration Measurements	3.9×10^{-4}
Sensing Subsystem, 2.6×10^{-4}	
Electrodes, 1.8×10^{-4}	
Amplifier, $1.1.1 \times 10^{-5}$	
Analog to Digital Conversion, 6.8×10^{-5}	
Calibration Curve, 1.3×10^{-4}	
Correcting the Nutrient Concentration	2.62×10^{-6}
Replenishment Solution Concentration, 1.98×10^{-9}	
Pumping Rate, 2.6×10^{-6}	
Random Pumping Error, 7.8×10^{-10}	
Pumping Offset, 2.0×10^{-11}	
Controlling the Volume of Nutrient Solution	7.8×10^{-6}
Total Error, σ_c^2	5.0×10^{-4}

Table 3.2 Sources of error in uptake measurements.

Source of error	Variance
Mixing Ratio	2.2×10^{-6}
Concentration Measurements,	1.9×10^{-4}
Sensing Subsystem, 1.3×10^{-4}	
Electrodes, 8.9×10^{-5}	
Amplifier, 5.7×10^{-6}	
Analog to Digital Conversion, 3.4×10^{-5}	
Calibration Curve, 6.4×10^{-5}	
Correcting the Nutrient Concentrations	2.6×10^{-6}
Replenishment Solution Concentration, 2.0×10^{-9}	
Pumping Rate, 2.6×10^{-6}	
Random Pumping Error, 7.5×10^{-10}	
Pumping Offset, 1.9×10^{-11}	
Estimate of Nutrients Added to Tank	5.3×10^{-4}
Replenishment Solution Concentration, 4.1×10^{-7}	
Pumping Rate, 5.3×10^{-4}	
Random Pumping Error, 7.5×10^{-8}	
Pumping Offset, 1.9×10^{-7}	
Initial Preparation of the Nutrient Solution	5.3×10^{-4}
Controlling the Volume of Nutrient Solution	1.4×10^{-4}
Total Error, σ_n^2	1.4×10^{-3}

Chapter 4 EXPERIMENTAL RESULTS

This chapter discusses the performance of the control and measurement system and the growth of plants in the Nutrient Flow System. The control and measurement are evaluated on the basis of the performance measures defined in Chapter 3; the variance of nutrient ion concentration and the error in uptake measurements.

In a controlled concentration system, maintaining a constant nutrient availability to the plants requires that as plants grow their roots are able to absorb more nutrients, as they would in their natural environment. The growth of plants is evaluated by comparing their growth to the exponential growth expected of young plants.

4.1 Performance of the Nutrient Flow System

Plots of measured concentration vs. time are given for ammonium, hydrogen ion, nitrate, and potassium. The average concentration, the square root of the variance, and the standard error of the mean are given for each ion. The plots of nitrate, hydrogen ion, and potassium concentration are from an experiment in which barley (*Hordeum vulgare*) was grown for sixteen days. The plot of ammonium concentration is from an experiment in which cottongrass (*Eriophorum vaginatum*) was grown for 10 days.

The standard error of the mean concentration is given for comparison with the results reported by Clement *et al.* [1974] and Woodhouse *et al.* [1978]. Standard error is normally used as an estimate of the variance of the mean of a subpopulation, and decreases as the size of the subpopulation increases.

In control systems, the amount of deviation from the target output is of as much interest as the average value of the controlled quantity. A concentration might be zero for the first half of an experiment and twice the target value for the second half. The average would be the target value, but this would not be a desirable result. The variance or the root mean squared error of the concentration are better performance measures because the performance of systems can be compared regardless of the number of measurements taken.

From chapter 3 (p. 69), the expected value of the variance of the concentration of an ion measured with a ion-specific electrode, σ_{q_e}, is 2.6% of the target concentration if 5% hysteresis is allowed in the control loop. The concentration of ammonium measured by the Nutrient Flow System is shown in Figure 4.1. In this experiment, the variance of the ammonium concentration, $\sigma_{NH_4^+}$, is 6.2% of the target concentration. The higher-than-expected variance is due to degraded performance (of the ammonia gas sensing electrode) caused by clogging of the gas permeable membrane.

The sawtooth shape of the nitrate concentration plot (Figure 4.2) is a result of hysteresis built into the control algorithm; the concentration is not corrected until there is at least a 5% error. This was done so that the amount of replenishment solution added could be measured more accurately as explained in chapter 3. The standard error (1.1×10^{-7}) is an order of magnitude smaller than that reported by Clement *et al.* [1974]. This is partly because twenty times as many measurements were made. Using the degrees of freedom reported by Clement *et al.* to calculate the variance of their results shows our Nutrient Flow System has about half the variation in nitrate concentration, even with the built-in hysteresis.

The nitrate electrode performed better than advertised by its manufacturer; the variance of the nitrate concentration was 2.45% of the target concentration, less than the 2.6% predicted by the error analysis of chapter 3.

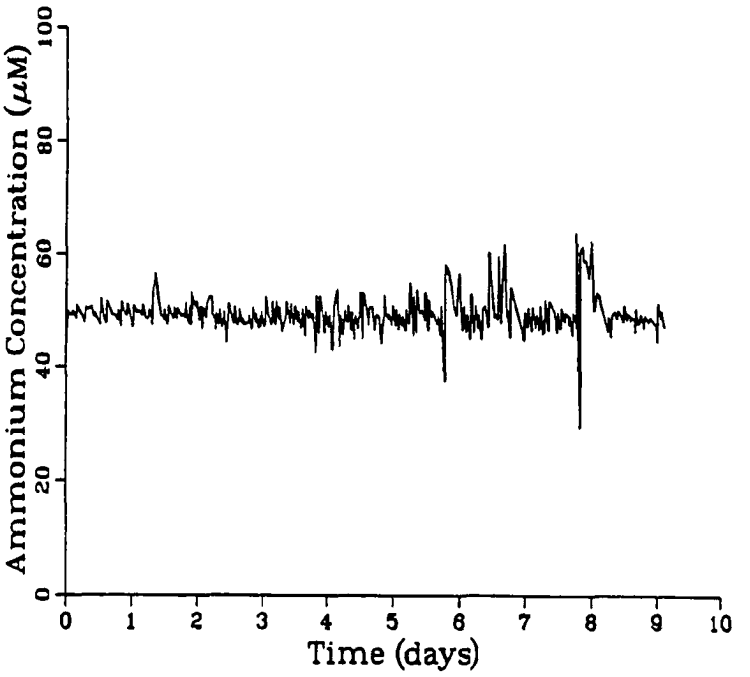

Figure 4.1 Ammonium concentration during nine days of growing cotton grass.

Hydrogen ion concentration was recorded during the experiment shown in Figure 4.2. Simultaneous plots of controlled hydrogen ion, nitrate, and potassium concentration have not, to my knowledge, been published elsewhere.

Like Woodhouse *et al.* [1978], I found that the potassium electrode only worked for about two weeks at a time. Bacteria grew on the sensing element, and could damage it. The nutrient flow system has been redesigned and rebuilt to allow preservative solutions to clean the potassium electrode when it is not being used. An example of potassium concentration vs. time is shown in Figure 4.2.

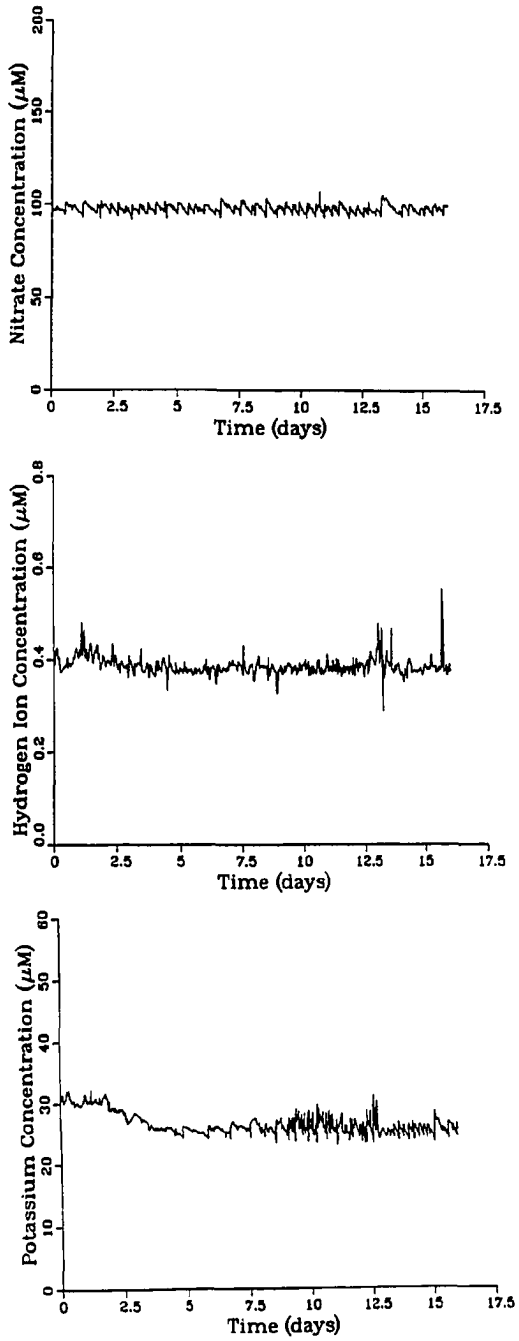

Figure 4.2 NO_3^-, H^+, and K^+ concentrations during sixteen days of growing barley.

The variance of the potassium concentration was higher than expected (σ_{K+} = 7.81% of the concentration). As with the ammonia electrode, performance deteriorated over the course of the experiment. The variation in the measured concentration increased until day 13, when the electrode was cleaned. After cleaning the electrode performed better.

Figure 4.3 shows the measured uptake for the experiment in which barley was grown for sixteen days. The uptake and concentration curves for nitrate and potassium ions shown in Figure 4.3 reflect a 5% hysteresis in the control loop. Ammonium uptake was not included because the pH of the nutrient solution affects the balance between ammonium and dissolved ammonia in solution. Dissolved ammonia may escape from the growing tank into the atmosphere, so measurement of ammonium uptake is inaccurate. The rate of volatilization has not been characterized.

Nitrate uptake (Figure 4.3) measurements were 1.5% higher than the amount of nitrate missing from the reservoirs of replenishment solutions.

The discrepancy between the potassium uptake measured as shown in Figure 4.3 and the potassium missing from the replenishment solution reservoirs was 10%. The error is due to poor electrode performance.

These results indicate that the Nutrient Flow System can measure nitrate uptake with more than adequate accuracy for most ecological investigations. Ammonium concentration control and potassium concentration control and uptake measurements may be sufficiently accurate for many experiments. With the additional refinements made to the system, these measurements should be more accurate.

4.2 Plant Growth

Before the Nutrient Flow System could be used to examine plant growth under a wide variety of conditions with several species, it was necessary to confirm that the plants would

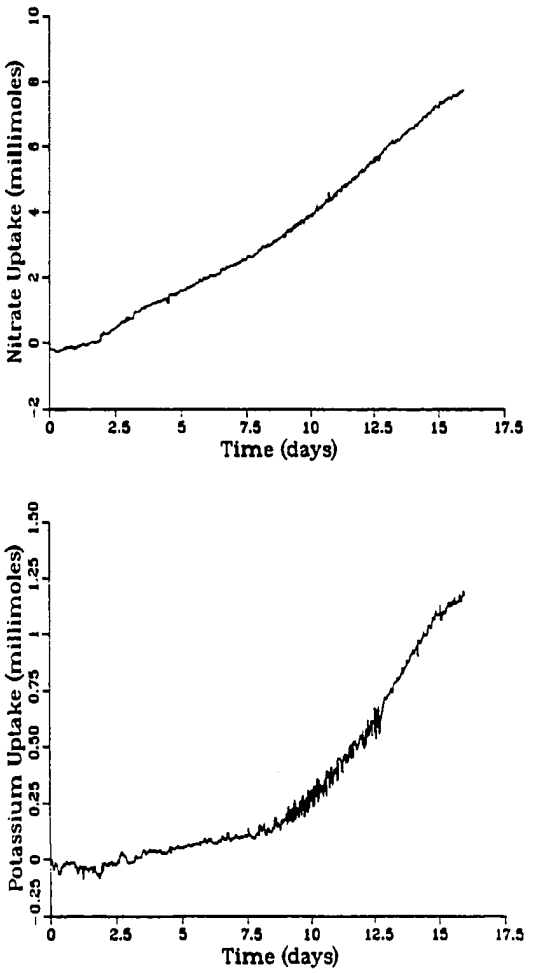

Figure 4.3 Nitrate and Potassium uptake over a sixteen day experiment.

grow normally in the system under optimum conditions. Barley was the species chosen for these tests.

Barley seeds were sprouted between two pieces of cheesecloth on a stainless steel wire mesh over aerated distilled water. After one week they were put into 1 inch diameter polyethylene bottle stoppers that had the bottoms cut out and replaced with screens. Seventy polyethylene stoppers, with plants, were then inserted into holes in the cover of the growing tank. The tank operated in a controlled environment room at a temperature of 20°C. The nutrient solution was held at 15°C, and the photoperiod was 20 hours.

Eight plants were harvested at the time of transplanting, and about every three days another sample of eight plants was harvested. The plants were rinsed, separated into roots and shoots, dried, and weighed. The natural logarithm of the mean weight of each harvest was plotted against the time of harvest.

4.3 Interpretation of Experimental Results

The tests of operation of the Nutrient Flow System used young plants that could be expected to grow exponentially. If the plants naturally grow exponentially and the nutrients available to them are proportional to the size of the plants, then a plot of log of plant weight vs. time should give a straight line, with a slope equal to the relative growth rate of the plants.

Table 4.1 summarizes the results of trials of plant growth performed during the development of the Nutrient Flow System. Figure 4.4 shows the growth of plants in experiment 1 of Table 4.1.

Table 4.1 *Growth rate of barley plants.*

Experiment	NO_3^- Concentration	Growth Rate	Standard Error
1	0.1 mM	0.144	0.039
2	0.1 mM	0.151	0.048
3	0.01 mM	0.076	0.045
4	0.01 mM	0.108	0.053

To test the assumption that the plants grew exponentially, a test for linearity was used [Zar 1984]. This test compares the variability among plants in a harvest to the variability between harvests. The growth of the barley plants was nonlinear ($P < 0.05$). Possible causes for the nonlinearity are: 1) the plants were not sampled strictly randomly, 2) the roots may not have received proportionately as much spray as they grew below the level of the spray nozzles, 3) the plants may not have used up their seed reserves completely, or 4) the barley plants may not grow at a constant rate over an extended period.

If growth had been linear, the effects of treatments could have been compared by using the Student's t-test (for two experiments) or analysis of covariance and multiple comparisons test (more than two experiments) to compare the slopes of the fitted curves [Zar 1984]. It is important to compare the slopes to ensure that differences in the final weight of seedlings do not reflect differences in initial seed size. Several harvests should be used to calculate the slopes because: 1) Just using initial and final weight does not make use of as much information as fitting a curve to the data and does not necessarily give the same growth rate. 2) Several harvests are necessary to check for linearity. If the growth rate is not constant throughout an experiment, it may be difficult to compare experiments.

Comparing relative growth rates rather than final weights is even more important when comparing adaptations of different species to limited nutrient supply. Any two species are not likely to have identical seed weights, so it is essential to test their relative growth in

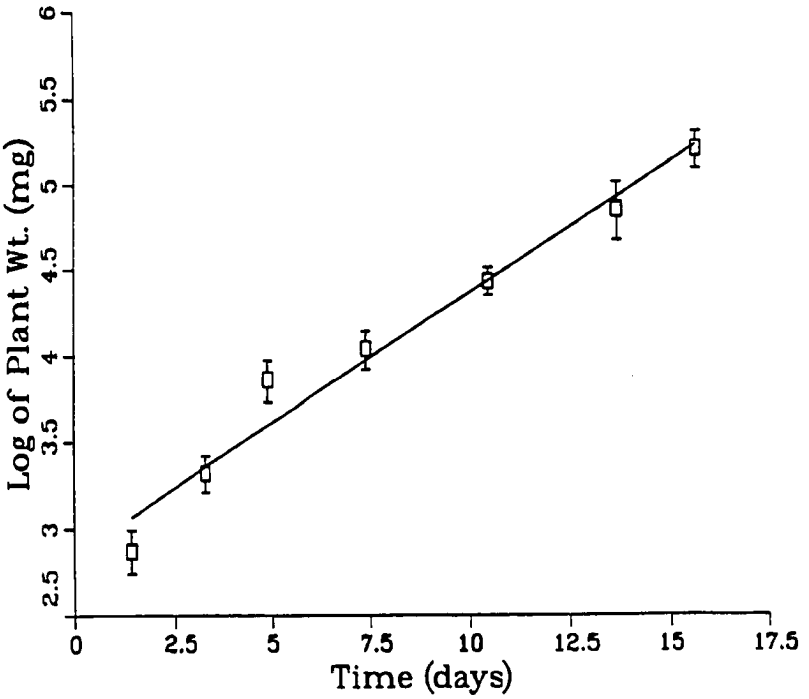

Figure 4.4 Expt. 1: Log of barley weight vs. time with 95% c.i. and linear fit.

an experiment, and compare it using the Student's t-test or analysis of covariance with multiple comparisons. (Easily calculated using technique, for example, of Zar [1984])

Chapter 5 CONCLUSIONS

There have been several controlled concentration flowing culture systems built, but the Nutrient Flow System is the first to have been analyzed using the standard mathematical tools available for systems analysis. Application of these tools allows estimation of the system's performance, and improves understanding of how the parameters of the system affect its performance. Most published reports on systems designed to measure or control nutrient concentrations simply describe what happened when certain components were put together, with little discussion of the sources of error that were introduced or the sensitivity of the system to these errors.

The mathematical analysis (chapter 3) provided valuable information as to what choices of various parameters would give accurate measurements. With appropriate choices of parameters, the performance of the Nutrient Flow System is primarily limited by the reliability of the chemical analysis. The measured concentration is estimated to have a root mean square deviation of 2.2% of the target concentration, and the estimated root mean square error of uptake measurements is 3.7% of the total uptake. For typical experiments the model predicted that electrode error, digitization error, and pumping rate error would contribute 54%, 20%, and 18% of the error in concentration control, respectively. The concentration measurements, pumping rate error, initial preparation of the nutrient solution, and variation in volume of nutrient solution would account for 14%, 38%, 38%, and 9% of the error in uptake measurements, respectively. In actual experiments, the nitrate electrode performed better than the model, but the potassium and ammonium electrodes performed worse. Improving the reliability of the chemical analyses would be the best way of obtaining better performance from the Nutrient Flow System.

Of course it is ecologists, for whom the system was designed, who must decide how accurate it needs to be. For most ecological investigations, improving the electrode reliability, adding more sensors, and improving the chemical analysis techniques will provide a sufficiently accurate system.

Some of the questions about future directions for development of the flowing solution culture systems should be addressed by ecologists. Most of the literature on flowing solution culture does not address the question of exactly what nutrient availability means. It could be defined as a flux, in units of moles per unit volume root space per second. Some have chosen (implicitly) to define it as moles per plant per unit time (Ingestad and Lund [1986]), or concentration (Hatch *et. al* [1986].) The best definition may depend on the application, but in any case, the definition should be explicitly stated and justified.

The application of a statistically sound, standard technique for evaluating the results of plant growth experiments would make comparisons between experiments done with different systems easier.

From an engineer's point of view the Nutrient Flow System is a nonlinear, multivariate, non-zero set point regulator problem for a "plant" with time-varying parameters, noisy detectors, and imperfect control actions. These characteristics made the design of the Nutrient Flow System interesting and challenging. Developing a model to better describe these characteristics would provide interesting material for further study. The analysis in this thesis was designed (through notation and technique) to provide a foundation that could be expanded with more advanced techniques.

Appendix A Derivation of the Concentration Output Variance

The electrode output voltage equation,

$$V_x = S \log x + V_0$$

can be solved for the concentration,

$$x = 10^{\left(\frac{V_x - V_0}{S}\right)}.$$

Where

$$S = \frac{-V_2 + V_1}{\log x_2 - \log x_1}$$

$$S = \frac{-V_2 + V_1}{\log a}$$

$$\log a = \log x_2 - \log x_1$$

$$\log a = \log \frac{x_2}{x_1}$$

$$a = x_2/x_1$$

$$V_0 = \frac{V_2 \log x_2 + V_1 \log x_1}{\log x_2 - \log x_1}$$

$$V_0 = \frac{V_2 \log x_2 + V_1 \log x_1}{\log a}.$$

Let $y = \frac{V_x - V_0}{S}$, and $x = 10^y$. Using the expression above, y can be found in terms of V_x, V_1,V_2, and a.

$$y = \frac{V_x - \frac{V_2 \log x_2 + V_1 \log x_1}{\log a}}{\frac{-V_2 + V_1}{\log a}}$$

$$y = \frac{V_x \log a - (V_2 \log x_2 + V_1 \log x_1)}{-V_2 + V_1}$$

but

$$(V_2 \log x_2 + V_1 \log x_1) = V_1(\log a + log x_1) - V_2 \log x_1$$

$$(V_2 \log x_2 + V_1 \log x_1) = V_1 \log a + (V_1 - V_2) \log x_1$$

so

$$y = \frac{V_x \log a - (V_1 \log a + (V_1 - V_2) \log x_1)}{V_1 - V_2}$$

$$y = \frac{V_x \log a}{V_1 - V_2} - \frac{V_1 \log a}{V_1 - V_2} - \log x_1.$$

To determine the effect of the variations in V_x, V_1, and V_2 on y, I find the partial derivatives with respect to V_1, V_2, and V_x.

$$\frac{\partial y}{\partial V_1} = \frac{-V_x \log a}{(V_1 - V_2)^2} - \left[\frac{\log a(V_1 - V_2) - (+V_1 \log a)(1)}{(V_1 - V_2)^2}\right]$$

$$\frac{\partial y}{\partial V_1} = \frac{-V_x \log a - V_1 \log a + V_2 \log a + V_1 \log a}{(V_1 - V_2)^2}$$

$$\frac{\partial y}{\partial V_1} = \frac{(V_2 - V_x) \log a}{(V_1 - V_2)^2}$$

$$\frac{\partial y}{\partial V_2} = \frac{-V_x \log a(-1)}{(V_1 - V_2)^2} - \frac{(-V_1 \log a)(-1)}{(V_1 - V_2)^2}$$

$$\frac{\partial y}{\partial V_2} = \frac{V_x \log a - V_1 \log a}{(V_1 - V_2)^2}$$

$$\frac{\partial y}{\partial V_2} = \frac{(V_x - V_1) \log a}{(V_1 - V_2)^2}$$

$$\frac{\partial y}{\partial V_x} = \frac{\log a}{V_1 - V_2}$$

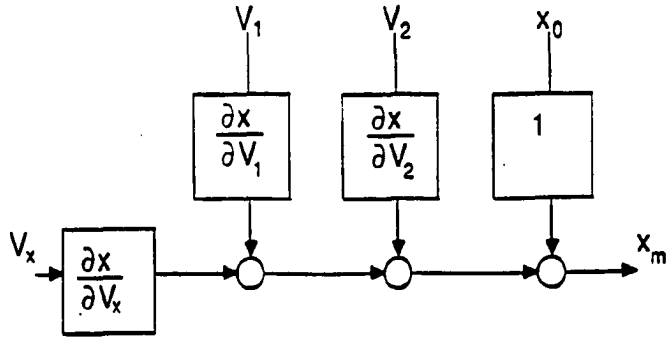

Figure A.1 Block diagram of the linear model for calculation of concentration.

Knowing partials of y, the partial derivatives of the measured concentration, x, can be found by using the chain rule for differentiation and

$$\frac{\partial a^{u(t)}}{\partial t} = (a^{u(t)} \ln a)\frac{\partial u(t)}{\partial t}.$$

The partial derivatives are

$$\frac{\partial x}{\partial V_1} = \frac{\partial 10^y}{\partial V_1} = \frac{10^y \ln 10 \log a(V_2 - V_x)}{(V_1 - V_2)^2}$$

$$\frac{\partial x}{\partial V_1} = \frac{x \ln 10 \log a(V_2 - V_x)}{(V_1 - V_2)^2}$$

$$\frac{\partial x}{\partial V_2} = \frac{x \ln 10 \log a(V_x - V_1)}{(V_1 - V_2)^2}$$

$$\frac{\partial x}{\partial V_x} = \frac{x \ln 10 \log a}{V_1 - V_2}.$$

The partial derivatives may be used to describe the response of the system near the operating point. A linear model to calculate the concentration of the tank solution is

$$x_m \approx \left(\frac{\partial x}{\partial V_x}\right)(V_x - V_{x_0}) + \left(\frac{\partial x}{\partial V_1}\right)(V_1 - V_{x_0}) + \left(\frac{\partial x}{\partial V_2}\right)(V_2 - V_{x_0}) + x_0.$$

These results may be used to calculate the variance of the measurements of ion concentration.

$$\sigma_x^2 = \left(\frac{\partial x}{\partial V_x}\right)^2 \sigma_{V_x}^2 + \left(\frac{\partial x}{\partial V_1}\right)^2 \sigma_{V_1}^2 + \left(\frac{\partial x}{\partial V_2}\right)^2 \sigma_{V_2}^2$$

$$\sigma_x^2 = (x \ln 10 \log a)^2 \left[\frac{\sigma_{V_x}^2}{(V_1 - V_2)^2} + \frac{(V_2 - V_x)^2 \sigma_{V_1}^2}{(V_1 - V_2)^4} + \frac{(V_x - V_1)^2 \sigma_{V_2}^2}{(V_1 - V_2)^4}\right].$$

This may simplified by using

$$V_1 - V_2 = S(\log x_2 - \log x_1) = S \log a$$

and

$$V_2 - V_x = S \log \frac{x_2}{x}$$

$$V_x - V_1 = S \log \frac{x}{x_1}.$$

$$\sigma_x^2 = (x \ln 10 \log a)^2 \left[\frac{\sigma_{V_x}^2}{(S \log a)^2} + \frac{(V_2 - V_x)^2 \sigma_{V_1}^2}{(S \log a)^4} + \frac{(V_x - V_1)^2 \sigma_{V_2}^2}{(S \log a)^4}\right]$$

$$\sigma_x^2 = (x \ln 10 \log a)^2 \left[\frac{\sigma_{V_x}^2}{(S \log a)^2} + \frac{(S \log(x_2/x))^2 \sigma_{V_1}^2}{(S \log a)^4} + \frac{(S \log(x/x_1))^2 \sigma_{V_2}^2}{(S \log a)^4}\right]$$

$$\sigma_x^2 = (x \ln 10)^2 \left[\frac{\sigma_{V_x}^2}{S^2} + \frac{(\log(x_2/x))^2 \sigma_{V_1}^2}{(S \log a)^2} + \frac{(\log(x/x_1))^2 \sigma_{V_2}^2}{(S \log a)^2}\right]$$

$$\sigma_x^2 = (x \ln 10)^2 \left[\frac{\sigma_{V_x}^2}{S^2} + \frac{(-\log(x/ax_1))^2 \sigma_{V_1}^2}{(S \log a)^2} + \frac{(x/x_1)^2 \sigma_{V_2}^2}{(S \log a)^2}\right].$$

Let $b = \frac{x}{x_1}$

$$\sigma_x^2 = (x \ln 10)^2 \left[\frac{\sigma_{V_x}^2}{S^2} + \frac{(-\log(b/a))^2 \sigma_{V_1}^2}{(S \log a)^2} + \frac{(\log b)^2 \sigma_{V_2}^2}{(S \log a)^2}\right].$$

The choice of b affects the variance, σ_x^2. The best choice of b can be found by minimizing $f(b)$ where

$$f(b) = (-\log(b/a))^2 \sigma_{V_1}^2 + (\log b)^2 \sigma_{V_2}^2$$

$$0 = \frac{\partial f(b)}{\partial b} = 2(-\log(b/a)) \left(\frac{-1}{(b/a)\ln 10} \right) (1/a)\sigma_{V_1}^2 + (2\log b)(1/b\ln 10)\sigma_{V_2}^2$$

$$0 = (\log b/a)(a/a)\sigma_{V_1}^2 + (\log b)\sigma_{V_2}^2$$

$$0 = (\log b - \log a)\sigma_{V_1}^2 + (\log b)\sigma_{V_2}^2$$

$$\sigma_{V_1}^2 \log a = (\sigma_{V_1}^2 + \sigma_{V_2}^2) \log b$$

$$\log b = \left(\frac{\sigma_{V_1}^2}{\sigma_{V_1}^2 + \sigma_{V_2}^2} \right) \log a.$$

The best choice of b is

$$b = a^{\sigma_{V_1}^2/(\sigma_{V_1}^2 + \sigma_{V_2}^2)}$$

Let $\gamma = \left(\frac{\sigma_{V_1}^2}{\sigma_{V_1}^2 + \sigma_{V_2}^2} \right)$, then

$$\sigma_x^2 = (\frac{x \ln 10}{S})^2 \left[\sigma_{V_x}^2 + \frac{(-\log(a^\gamma/a))^2 \sigma_{V_1}^2}{(\log a)^2} + \frac{(\log a^\gamma)^2 \sigma_{V_2}^2}{(\log a)^2} \right]$$

$$\sigma_x^2 = (\frac{x \ln 10}{S})^2 \left[\sigma_{V_x}^2 + \frac{(\log a - \gamma \log a)^2 \sigma_{V_1}^2}{(\log a)^2} + \frac{(\gamma \log a)^2 \sigma_{V_2}^2}{(\log a)^2} \right]$$

$$\sigma_x^2 = (\frac{x \ln 10}{S})^2 \left[\sigma_{V_x}^2 + (1-\gamma)^2 \sigma_{V_2}^2 + \gamma^2 \sigma_{V_2}^2 \right]$$

$$1-\gamma=\left(\frac{\sigma_{V_1}^2+\sigma_{V_2}^2-\sigma_{V_1}^2}{\sigma_{V_1}^2+\sigma_{V_2}^2}\right)$$

$$1-\gamma=\left(\frac{\sigma_{V_2}^2}{\sigma_{V_1}^2+\sigma_{V_2}^2}\right)$$

$$\sigma_x^2=(\frac{x\ln 10}{S})^2\left[\sigma_{V_x}^2+\left(\frac{\sigma_{V_2}^2}{\sigma_{V_1}^2+\sigma_{V_2}^2}\right)^2\sigma_{V_1}^2+\left(\frac{\sigma_{V_1}^2}{\sigma_{V_1}^2+\sigma_{V_2}^2}\right)^2\sigma_{V_2}^2\right].$$

It is expected that $\sigma_{V_1}^2=\sigma_{V_2}^2$, so the noise introduced is

$$\sigma_x^2=(\frac{x\ln 10}{S})^2\left[\sigma_{V_x}^2+\frac{1}{4}\sigma_{V_1}^2+\frac{1}{4}\sigma_{V_1}^2\right],$$

and the best choices of x_0, x_1, and x_2 are such that $x_0=\sqrt{x_1x_2}$.

REFERENCES

Asher, C. J., P. G. Ozane, and J. F. Loneragan (1965) A method for controlling the ionic environment of plant roots. *Soil Sci.*, **100**: 149-156

Bentley, J. P. (1983) *Principles of Measurement Systems*, Longman Inc., N.Y.

Ben-Yaakov, S., and J. Ben-Asher (1982) System design and analysis of continuous monitoring of the environment in nutrient solution culture. *J. Plant Nutr.*, **5**: 45-55

Blom-Zandstra, M., and G. L. Jupijn (1987) A computer-controlled multi-titration system to study transpiration, OH^- efflux and nitrate uptake by intact lettuce plants (*Lactuca sativa L.*) under different environmental conditions. *Plant Cell Environ.*, **10**: 545-550

Bloom, A. J. (1989) Continuous and Steady-State Nutrient Absorbtion by Intact Plants. In *Applications of Continuous and Steady-State Methods to Root Biology.*, pages 147-163 Kluwer Academic Pub., Dordrecht

Bloom, A. J., and F. S. Chapin, III (1981) Differences in steady-state ammonium and nitrate influx by cold and warm adapted barley varieties. *Plant Physiol.*, **68**: 1064-1067

Bloom, A. J., H. A. Mooney, O. Bjorkman, and J. Berry (1980) Materials and methods for carbon dioxide and water exchange analysis. *Plant Cell Environ.*, **3**: 371-376

Breeze, V. G., R. J. Canaway, A. Wild, M. J. Hopper, L. H. P. Jones (1982) The uptake of phosphate by plants from flowing nutrient solution. *J. Expt. Bot.*, **33**: 183-189

Caldwell, C. D., P. E. LeFevere, and D. P. Aikman (1978) An open-circuit apparatus for continuous determination of net uptake by seedlings grown hydroponically. *Can. J. Bot.*, **56**: 2767-2772

Clement, C. R., M. J. Hopper, R. J. Canaway, L. H. P. Jones (1974) A system for measuring the uptake of ions by plants from flowing solutions of controlled composition. *J. Expt. Bot.*, **25**: 81-99

Epstein, E. (1972) *Mineral Nutrition of Plants: Principles and Perspectives.*, John Wiley and Sons, Inc., N.Y.

Glass, A. D. M., M. Y. Siddiqi, C. E. Deane-Drummond (1987) A microcomputer based system for continuously measuring and recording ion activities of uptake solutions during ion absorbtion by roots of intact plants. *Plant Cell Environ.*, **6**: 247-253

Goyal, S. S., and R. C. Huffaker (1986) The uptake of NO_3^-, NO_2^-, and NH_4^+ by intact wheat (*Triticum aestivum*) seedlings: I. Induction and kinetics of transport systems. *Plant Physiol.*, **82**: 1051-1056

Hatch, D. J., M. J. Hopper, and M. S. Dhanoa (1986) Measurement of ammonium ions in flowing solution culture and diurnal variation in uptake by *Lolium perenne L. J. Exp. Bot.*, **37**: 589-596

Hatch, D. J., and R. J. Canaway (1984) Control of pH in a system of flowing solution culture using a microcomputer. *J. Exp. Bot.*, **35**: 1860-1868

Ingestad, T. (1971) A definition of optimum nutrient requirements in birch seedlings. II *Physiol. Plant.*, **24**: 118-125

Ingestad, T. (1982) Relative addition rate and external concentration: driving variables used in plant nutrition. *Plant Cell Environ.*, **5**: 443-453

Ingestad, T. and A. Lund (1979) Nitrogen stress in birch seedlings: I. Growth technique and growth. *Physiol. Plant.*, **45**: 137-148

Ingestad, T. and A. Lund (1986) Theory and techniques for steady-state mineral nutrition and growth of plants. *Scand. J. For. Res.*, **1**: 439-453

Koch, G.W., W. E. Winner, A. Nardone, and H. A. Mooney (1987) A system for controlling the root and shoot environment for plant growth studies. *Environ. Exp. Bot*, **27**: 365-377

Kuo, B. C. (1982) *Automatic Control Systems*, Prentice-Hall, Inc., Englewood Cliffs, N.J.

Nye, P.H., and P. B. Tinker (1977) *Solute movement in the soil-root system.*, Univ. of California Press, Berkeley

O'Flynn, M., E. Moriarty (1987) *Linear Systems: Time Domain and Transform Analysis*, Harper & Row, Inc., N.Y.

Oppenheim, A.V., A. S. Willsky, and T. A. Young (1983) *Signals and Systems*, Prentice-Hall, Inc., Englewood Cliffs, N.J.

Orion Research, Inc. (1986) *Ammonia Electrode Instruction Manual*, 529 Main St., Boston, MA 02129

Orion Research, Inc. (1986) *Nitrate Electrode Instruction Manual*, 529 Main St., Boston, MA 02129

Orion Research, Inc. (1986) *Potassium Electrode Instruction Manual*, 529 Main St., Boston, MA 02129

Reisenauer, H. M. (1969) A technique for growing plants at controlled levels of all nutrients. *Soil Sci.*, **108**: 350-353

Van De Dijk, S. J. (1981) Differences in nitrate uptake of species from habitats rich or poor in nitrogen when grown at low nitrate concentrations, using a new growth technique. *Plant Soil*, **62**: 265-278

Woodhouse, P. J., A. Wild, and C. R. Clement (1978) Rate of uptake of potassium by three crop species in relation to growth *J. Expt. Bot.*, **29**: 885-894

Zar, J. H. (1984) *Biostatistical Analysis*, Prentice-Hall, Inc., Englewood Cliffs, N.J.